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The early evolution of family life

Thomas Botterill-James, BA-BSc, BSc Hons

School of Natural Sciences, University of Tasmania

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Statement of co-authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Thomas Botterill-James: School of Natural Sciences, University of Tasmania
David G Chapple: College of Science and Engineering, Flinders University
Lucy Ford: Institute of Evolutionary Biology, University of Edinburgh
Michael G Gardner: School of Biological Sciences, Monash University
Ben Halliwell: School of Natural Sciences, University of Tasmania
Iain S Koolhof: School of Natural Sciences, University of Tasmania
Simon McKeown: School of Natural Sciences, University of Tasmania
Kirke L Munch: School of Natural Sciences, University of Tasmania
Dan WA Noble: Research School of Biology, Australian National University
Jacinta Sillince: School of Natural Sciences, University of Tasmania
Per T Smiseth: Institute of Evolutionary Biology, University of Edinburgh
Tobias Uller: Department of Biology, Lund University
Erik Wapstra: School of Natural Sciences, University of Tasmania
Geoffrey M While: School of Natural Sciences, University of Tasmania

Paper One (Chapter Two): **Botterill-James T**, Sillince J, Uller T, Chapple DG, Gardner MG, Wapstra E and While GM (2017) Experimental manipulation suggests effect of polyandry but not mate familiarity on within-pair aggression in the social skink, *Liopholis whitii*. Published in *Behavioral Ecology and Sociobiology.*

TBJ, GW, EW, TU, DGC and MGG conceived of and designed the study. TBJ and JS caught field animals and recorded and scored interaction footage. TBJ analysed the data. TBJ, GW and TU drafted the manuscript with input from all other authors. All authors gave approval for publication and agreed to be held accountable for the content therein.

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We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or in preparation) peer-reviewed manuscripts contributing to this thesis:

Signed:

Geoffrey While
Candidate's supervisor
School of Natural Sciences
University of Tasmania
Date: 28/06/2019

Signed:

Erik Wapstra
Candidate's supervisor
School of Natural Sciences
University of Tasmania
Date: 28/06/2019

Signed:

Greg Jordan
Head of Discipline of Biological Sciences
School of Natural Sciences
University of Tasmania
Date: 28/06/2019

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ABSTRACT

Abstract

Family living is a key life history characteristic of many species throughout the animal kingdom and is a fundamental early step in the 'major evolutionary transition' to eusociality. To date, most research on family living has focussed on (1) the role of parent-offspring interactions in the maintenance of family groups, and (2), studied such interactions in highly complex social systems that are often found in birds, mammals and eusocial insects. As a consequence, there is currently an incomplete understanding of the processes responsible for the evolutionary emergence and maintenance of simple family groups that form the platform for the evolution of more complex social systems. My thesis uses a family-living lizard, *Liopholis whitii*, that displays long-term, stable male-female pair bonds and prolonged parent-offspring associations to provide insights into this knowledge gap. Specifically, I quantify the extent of conflict between different dyads within families (i.e., compare levels of sexual, parent-offspring and sibling conflict), and experimentally show how female mating patterns and food availability influence the extent of conflict between different family dyads and the consequences for family structure. I show (1) that conflict is high between monogamous pairs, fathers and offspring, and between siblings, but virtually non-existent between mothers and offspring, (2) that conflict is increased between monogamous pairs when females undertake extra-pair matings, and (3) that there is no effect of food availability or sibling relatedness on sibling conflict. Combined with previous research in this species that demonstrates high paternal aggression towards extra-pair offspring, these results suggest that genetic monogamy, but not food availability, is crucial to stabilising family life in *L. whitii*, by reducing conflict between monogamous pairs and between fathers and offspring. I complemented these experiments on *L. whitii* with an investigation of how genetic relatedness and food availability shape sibling conflict in another facultatively family living species, the burying beetle *Nicrophorus vespillloides*. In contrast to the lizards, I found no effect of genetic relatedness, but an effect of food availability, on the level of begging between siblings. Finally, I investigated how parental effects mediate conflict between family members in a more indirect and complex manner. Specifically, I used a meta-analytical and comparative approach across bird species to examine the consequences of a parentally controlled trait, asynchronous hatching of offspring, for the fitness of different family members. I find evidence that hatching asynchrony benefits

parents by reducing their parental effort, but at a cost to last hatched offspring. Overall, my thesis highlights that examining all dyadic relationships within simple family groups and incorporating parental effects is crucial for research that aims to build a detailed and holistic picture of the early evolution of family life, and subsequent evolution of more complex sociality.

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CHAPTER ONE

General Introduction

Evolution of egalitarian and fraternal societies

Sociality is a captivating aspect of animal life, encompassing a variety of fascinating behaviours. Social groups range from transient associations between breeding adults, to mating partnerships maintained across years, stable family groups with or without overlapping generations, and, in extreme cases, complex eusocial colonies featuring morphologically distinct castes, sterile workers and intricately specialised divisions of labour (Krause and Ruxton 2002). Such groups emerge from non-random associations between unrelated conspecifics (egalitarian societies) or from family members remaining together (fraternal societies) (Queller 2000). The evolution of highly complex behaviours and societies characteristic of eusociality has occurred multiple times independently; eight times in hymneoptera (Hughes et al. 2008), twice in mammals (both in mole-rats; Jarvis and Bennet 1993) and at least three times in snapping shrimp (Duffy et al. 2000) and has been highlighted as a major evolutionary transition (sensu Maynard-Smith and Szthmary 1995; West et al. 2015). Thus, finding generalised explanations for the evolutionary origins and diversification of animal societies represents a significant challenge for evolutionary biologists.

At the basis of both egalitarian and fraternal societies are cooperative behaviours – that is behaviours that provide a benefit to another individual and that have evolved, at least partially, because of this benefit (West et al. 2007). These range from simple tolerance of conspecifics at a shared resource (e.g., Chapple 2003; Dickinson and McGowan 2005), to vigilance and shared defensive herding behaviours (e.g., Griesser and Ekman 2005), through to helping others reproduce (e.g., Koenig and Dickinson 2004; Clutton-Brock 2006). Explaining the evolution of animal societies therefore requires explaining the cooperative behaviours that underpin them. However, cooperative behaviours and the genes coding for them pose a problem for evolutionary theory as – all else being equal – they typically reduce the relative fitness of an individual performing them and hence should be selected against and not spread in a population. Finding fitness benefits of cooperative behaviours for the individuals performing them is hence fundamental for biologists trying to explain the evolution of animal societies.

Animal societies can evolve when cooperation provides direct fitness benefits to the individuals performing the cooperative behaviours. The simplest way this can occur is when individuals perform behaviours that enhance their own fitness, but also have a side or 'by-product' effect of enhancing the fitness of other group members. For example, in many cooperatively breeding vertebrates, individuals are selected to tolerate (or even help rear) offspring that are not their own, as this increases group size and larger group size then has fitness benefits for the whole group through enhanced foraging/hunting success (e.g., in wild dogs; Creel and Creel 1995), likelihood of winning conflicts with other groups (e.g., in meerkats; Clutton-Brock 2009, in primates; Wrangham 1980), or ability to reduce predation (e.g., mobbing behaviour in birds; Andersson and Wiklund 1978) ('group augmentation'; Kokko et al. 2001). The second key way cooperation can provide an individual with direct fitness benefits is when there is a mechanism for enhancing reciprocation of cooperative behaviours, either by rewarding co-operators or punishing cheaters (Trivers 1971; Axelrod and Hamilton 1981; Frank 2003). Trivers (1971) emphasised that cooperation could be favoured in reciprocal interactions, by individuals preferentially aiding those that have helped them in the past, or by helping those that help others. There are many empirical examples of cooperative reciprocation in animals, including reef fish interactions with cleaner fish (Bshary and Schäffer 2002), allo-grooming in primates (Barret et al. 1999; Schino and Aureli 2009), and food-sharing in rats (Schweinfurth and Taborsky 2018). In addition, much of the complex cooperation displayed by humans is underpinned by reciprocity (Fehr and Fischbacher 2003). In such cases, cooperation provides a direct fitness benefit, and is only favoured if the short term cost of being cooperative is outweighed by the long term benefit of receiving cooperation (Lehmann and Keller 2006).

In contrast to these examples, individuals can also behave in a way that appears altruistic; that is, perform a behaviour that provides no obvious direct benefit to themselves (or even comes at a significant personal cost) but that provides a direct benefit to another individual. The evolution of these types of cooperative behaviours require a different explanation; namely, kin selection. Kin selection works such that individuals gain fitness benefits from a given trait, but indirectly, by cooperating preferentially with individuals who also carry a copy of the gene for the given trait (Hamilton 1964). The simplest way this process can occur is if cooperation is directed

towards relatives, and therefore kin selection in often invoked to help explain the evolution of fraternal societies (Maynard-Smith 1964). By helping a direct relative to reproduce, an individual can act to maximise the number of copies of their genes they pass to the next generation, albeit indirectly; these individuals might not maximise their fitness directly by performing a costly cooperative behaviour, but by aiding relatives can instead maximise their 'inclusive fitness'. Therefore, kin selection can explain how costly cooperative behaviours and the gene/s underlying them can spread in a population (Hamilton 1964).

Hamilton's rule and the evolution of fraternal societies

A simple mathematical formula, Hamilton's inclusive fitness rule, simply and elegantly outlines that cooperation between relatives is expected to evolve when;

$$rB - c > 0$$

where r = the relatedness between an actor performing a cooperative behaviour and the recipient of that behaviour, B = the benefit of the cooperative behaviour to the recipient, and c = the cost of the cooperative behaviour to the actor. Putting this inequality into words, cooperation will be favoured if the benefits to the recipient (B), weighted by the genetic relatedness of the recipient to the actor (r), outweigh the costs to the actor (c). Two key predictions from this is that (1) cooperation between relatives will occur when genetic relatedness is sufficiently high, which will be the case in social systems with low rates of promiscuous mating (Boomsma 2007), and (2) cooperation between relatives will occur when the cost to benefit ratio of cooperation for the actor is low.

Hamilton's rule and its intuitive explanation of how costly cooperation can evolve has been one of the most fundamental advances in evolutionary theory since Darwin's theory of natural selection (Bourke 2011; Davies et al. 2012, but see Nowak et al. 2010; Allen et al. 2013; Nowak and Allen 2015; Nowak et al. 2017 for criticisms of Hamilton's rule, Abbot et al. 2011; Boomsma et al. 2011; Ferriere and Michod 2011; Herre and Wcislo 2011; Strassman et al. 2011 for replies, and Birch 2014; Birch and Okasha 2014; Kramer and Meunier 2016 for in-depth reviews of the debate). Consequently, Hamilton's rule and the concept of inclusive fitness has generated a huge number of empirical studies testing the ability of inclusive fitness theory to predict patterns of

sociality in nature, with great success (reviewed in Abbot et al. 2011; Bourke 2014; Rubenstein and Abbott 2017). Evidence for the importance of Hamilton's rule in explaining cooperative behaviour comes from two lines of empirical evidence. First, within species, experimental and observational studies have demonstrated that individual facultative adjustments in cooperative behaviour are dependent on both relatedness between individuals and the costs and benefits of cooperative behaviour (reviewed in Bourke 2014). For example, a large meta-analysis of facultative adjustment of paternal care in response to paternity loss across a range of vertebrate and invertebrate taxa demonstrated that males facultatively reduced paternal investment in response to paternity loss in cases where paternal care is costly (Griffin et al. 2010). Second, between species, comparative analyses have shown that high levels of genetic relatedness between family members has been crucial to the formation of fraternal societies across a range of taxa; low levels of promiscuity are strongly associated with evolutionary transitions from family to cooperative breeding in both birds and mammals (Cornwallis et al. 2010; Lukas and Clutton-Brock 2012), and each of eight independent transitions to eusociality in insects was preceded by genetic monogamy (Hughes et al. 2008; Boomsma et al. 2011).

The combination of the strong body of empirical literature supporting the predictions of Hamilton's rule, along with its intuitive appeal, has been fundamental to our current understanding of the evolution of fraternal societies. Despite this, there is a lack of empirical evidence and hence a thorough understanding of the evolution of these societies in three key areas.

Knowledge gaps in the evolution of fraternal societies

(1) Overemphasis on the *r* term

There has been on overemphasis (both in theoretical and empirical studies) on the *r* term in Hamilton's rule (Griffin and West 2002; West et al. 2007). This has been to the relative neglect of simultaneously testing how the *B* and *c* terms affect cooperative behaviour; that is, how the costs and benefits of cooperative behaviour shape their evolution. This is perhaps because of the relative ease in defining and quantifying genetic relatedness compared to doing the same for the costs and benefits of cooperative behaviours. Regardless of the reasons for overlooking the *B* and *c* terms of

Hamilton's rule, doing so can cause some confusion when trying to understand the causes of cooperation and altruism. For example, inclusive fitness theory predicts males should facultatively reduce paternal care in response to loss of paternity, but over 40 years of empirical research failed to find this effect, with a few exceptions (reviewed in Alonzo 2010). With a meta-analytical approach that accounted the costs of care, however, Griffin et al. (2010) found that in species where the costs of providing care were high, males did reduce care in response to paternity loss (or cues of paternity loss). More generally, where studies have explicitly considered the costs and benefits of cooperation, they have been shown to be important in mediating levels of cooperation and conflict between family members (reviewed in Bourke 2014). A clear example of the importance of *B* and *c* for mediating cooperation comes from the hairy-faced hover wasp, where females form an ordered queue for reproduction in the nest (Field et al. 2006). The dominant female lays eggs and the queueing subordinates help by foraging for food and bringing it back to the nest. In an experiment that removed high ranked subordinates from the nest (hence increasing the relative costs of helping for subordinates, as their chances of breeding were increased, and energy spent on foraging could reduce their energy available for breeding), lower ranked subordinates reduced their amount of helping (Field et al. 2006), in line with predictions from kin selection theory (Hamilton 1964; Cant and Field 2001). Such work demonstrates the value of explicitly considering the costs and benefits of social behaviours – not just relatedness between the individuals performing the behaviours – for understanding their evolution.

(2) Narrow dyadic focus in behavioural studies

Studies investigating facultative adjustment in cooperative behaviour in response to variation in genetic relatedness between relatives and/or the costs and benefits of cooperation have typically focussed on parental investment into offspring, with less focus on other processes occurring in families such as sibling interactions, parent-offspring competition (*sensu* Kramer et al. 2017) and offspring assistance to parents (reviewed in Kramer and Meunier 2018, but see Dreiss et al. 2010; Meunier and Kölliker 2012; Yip and Rayor 2013; Falk et al. 2014; Kramer et al. 2015; Schrader et al. 2015; Jarrett et al. 2017; Kramer et al. 2017). This can lead to an incomplete or even misleading picture of the evolution of families and complex fraternal societies (e.g., extended family groups/cooperatively breeders and eusocial societies). For example, in

the European earwing *Forficula auricularia*, offspring can accrue indirect fitness benefits by feeding siblings whom they are related to (Falk et al. 2014). These benefits may be more important than parental investment for some offspring; ignoring sibling cooperation here would result in a poor understanding of the benefits of family life, and therefore the ultimate causes of its evolution (Falk et al. 2014). Furthermore, most studies investigating facultative adjustment in cooperative behaviour in response to variation in the parameters of Hamilton's rule only focus on a single dyad at a time (Kramer and Meunier 2018; but see, for example, Smiseth et al. 2007; Schrader et al. 2015). This can hinder our understanding of the evolution of sociality (Kramer and Meunier 2018); using the earwig example above again, if we were to examine adjustment of paternal care of offspring in response to paternity loss, we might find no effect and conclude that promiscuity has no importance in shaping this social system and the interactions within it. However, we would come to the opposite conclusion if we were to expand our investigation beyond this dyad to also examine interactions between siblings.

In addition, broadening our focus away from single dyads may also allow us to examine the extent to which conflict and cooperation between different family members is mediated by more complex mechanisms. Specifically, we know that mothers (and fathers) can mediate levels of cooperation versus conflict within broods via parental effects. For example, in many bird species, parents can adjust the hatching span of their eggs through their incubation behaviour. This creates dominance hierarchies within the brood, which can then either exacerbate or minimise conflict between siblings over parental provisioning (Hahn et al. 1981; Roulin and Dreiss 2012) and modify parental versus last hatched offspring fitness (Stoleson and Beissenger 1995). This highlights that studying parental effects is important to fully understand the suite of mechanisms governing the evolution of social systems and the behaviours that characterise them (Royle et al. 2001; Paquet and Smiseth 2016; Kramer and Meunier 2018; Smiseth and Royle 2018).

(3) Taxonomic bias and neglect of the early evolution of family life

There has been a clear taxonomic bias towards understanding how Hamilton's rule operates in fraternal societies that are obligate and complex (Costa 2018; Kramer and

Meunier 2018; Kronauer and Libbrecht 2018). These societies include the cooperative breeding systems of many birds and mammals, where offspring delay dispersal to help raise their younger siblings, and even more complex eusocial insect societies where there is division of labour between workers and reproductive helpers. This bias has been at the relative neglect of testing if Hamilton's rule predicts cooperative behaviour in simpler facultative social systems, such as nuclear family units (Kramer and Meunier 2018; but see, for example, While et al. 2009a; Falk et al. 2014; Jarret et al. 2017). In these simple social systems, cooperation is characterised by basic forms of parental care (such as egg-guarding, or even simply tolerance of offspring within the parental home range) for non-overlapping generations of offspring (e.g., Botterill-James et al. 2016). These types of social systems are thought to be what occurs after the initial transition from solitary to social living and so represent the precursors to evolution of more complex societies (Queller 1994; Field and Brace 2004; Kramer and Meunier 2018). Ignoring how these systems evolve has led to a relatively poor understanding the origins of fraternal societies relative to their maintenance and diversification (Smsieth et al. 2012; Royle et al. 2016; Costa 2018; Kramer and Meunier 2018)

The factors driving the emergence of sociality are often not the same as those that are involved in their maintenance and subsequent diversification (Smiseth et al. 2003; Smiseth et al. 2012; Royle et al. 2016), and so not incorporating an understanding of the factors driving the initial emergence of fraternal societies can also cause an incomplete and even misleading understanding of complex sociality how evolves. For example, many comparative analyses have aimed to understand the ecological drivers of the evolution of cooperative breeding systems in birds, with some suggesting that stable, productive environments promote their evolution, while other studies instead suggest that unpredictable, harsh environments promote their evolution (stable, productive environments: Covas and Griesser 2007; Gonzalez 2013, unpredictable, harsh environments: Rubenstein and Lovette 2007; Jetz and Rubenstein 2011). A recent analysis by Griesser et al. (2017) separated out species into either family living (defined as species with prolonged parent-offspring associations beyond nutritional dependency) or cooperatively breeding (species with offspring retained at the nest and helping at the nest), finding that transitions to family living occurred readily in stable, productive environment, and transitions to cooperative breeding from family living

then occurred in unpredictable, harsh environments. Only by examining the selective forces promoting the evolution of more simple sociality were Griesser et al. (2017) able to clarify confusion over the ecological drivers of cooperative breeding in birds to provide an accurate understanding picture of the pathway from simple to highly complex fraternal societies.

Thesis aims

Here, using three different study systems (details below), I aim to provide empirical insights into the above knowledge gaps. I do this by using targeted experiments exploring how genetic relatedness and ecological factors influence conflict between family members in two social species that live in nuclear family units (representing what we might expect early in the evolutionary path towards more complex societies). Additionally, I conduct a series of meta-analyses and a comparative analysis across bird species to test how the costs and benefits of a maternal effect (hatching asynchrony) differ for parents versus offspring and between siblings, and hence the consequences of this maternal effect for family conflict. I also explore how these cost/benefits change with ecological conditions. Specifically, the set of integrated aims I test in this thesis are as follows:

1) Experimentally test how the extent of conflict varies between different dyads within families (i.e., compare levels of sexual, parent-offspring and sibling conflict)

2) Experimentally test the effects of genetic relatedness and the *Bc* terms of Hamilton's rule on the extent of within family conflicts

3) Experimentally test the effects of these factors on family structure via effects on offspring dispersal behaviour

4) Explore the ecological causes of hatching asynchrony and its consequences for family conflict

Study systems

White's skink

White's skink (*Liopholis whitii*) is a live-bearing (viviparous) skink that has a wide distribution throughout south-east Australia, including the island state of Tasmania. It is a member of the monophyletic *Egernia* group of skinks found throughout Australia. This group is comprised of approximately 60 species and seven genera. There is a wide range of social systems found within this group; some species live solitarily, some species form long term pair bonds without delayed offspring dispersal, and some display relatively complex social systems with extended multigenerational families containing up to 30 related individuals (reviewed in Chapple 2003; While et al. 2015; Gardner et al. 2016; Whiting and While 2017; While et al. 2019). *Liopholis whitii* display a social system that lies somewhere in the middle of this continuum of social complexity. Specifically, *L. whitii* live in kin groups characterised by stable social monogamy and prolonged association between one or two offspring often (out of a litter of 1-4) with their parents for 1-2 years, with one breeding season occurring per year (Chapple 2003; Chapple and Keogh 2006; While et al. 2009b). These associations are facultative, with variation in the presence versus absence and extent of parent-offspring associations occurring between individuals within a given year (While et al. 2009b; Botterill-James et al. 2016), and within individuals across years (While et al. 2009b). Offspring that do stay with their parents disperse prior to sexual maturity (3-4 years) of age to breed independently.

There are three other key biological traits that make *L. whitii* an ideal species for investigating how genetic relatedness and ecological factors shape the extent of conflict between family members. First, *L. whitii* exhibit levels of aggression towards one another that are (a) overt, (b) variable, and (c) easy to quantify (Sinn et al. 2008; McEvoy et al. 2013), allowing me to easily investigate the causal factors of variation in conflict between family members. Second, although exhibiting social monogamy, *L. whitii* do not exhibit strict genetic monogamy; they display moderate and variable (both within and between individual) rates of extra-pair mating, with approximately 35% of broods containing at least one extra pair offspring (While et al. 2009b). In addition, *L. whitii* individuals can discriminate between kin (While et al. 2009a; While et al. 2014)

potentially via olfaction (Bull et al. 2000), allowing me to examine how variation in genetic relatedness affects behaviour between family members. Third and finally, *L whitii* are easily observed and subject to experimental manipulation both in the field and the laboratory (e.g., Botterill-James et al. 2016; Halliwell et al. 2017a, b), taking exceptionally well to captivity where they show a behaviour that occurs naturally in the wild (Halliwell et al. 2017a, b). This allows me to examine behavioural responses of study animals to targeted manipulation of the parameters of Hamilton's rule, and interpret these responses in an ecologically relevant manner.

Burying beetle

Burying beetles are members of the coleopteran family Silphidae (the carrion beetles) of the genus *Nicrophorus.* There are 75 species in the genus, all living in the Northern Hemisphere (Royle et al. 2013). They are well known for exploiting small vertebrate carcasses, as are most other siphlids. But unlike other siphlids, who use vertebrate carrion primarily as an adult food source or somewhere to lay eggs, *Nicrophorus* beetles bury the carcass and provide extensive biparental care to their offspring before and after hatching (Pukowski 1933; Eggert and Muller 1997; Scott 1998). This parental care entails a number of pre- and post-hatching behaviours. Pre-hatching, parents will defend the carcass from conspecifics and other insects, bury the carcass and roll it into a ball, removing fur/feathers and apply oral and anal microbial secretions to the surface (Pukowski 1933; Suzuki 2001; Cotter 2010; Degenkolb 2011; Steiger 2011; Arce 2012). Mothers lay their eggs in the surrounding soil, and once larvae hatch they crawl to carcass. The larvae then aggregate at a small crater cut in the carcass by the parents that allows the larvae access to the carrion as a food source. The extent of post-hatching care varies between species, as does to the extent to which the male assists the female, but typically consists of continued parental maintenance of the carcass (rolling it into a ball and secreting antimicrobial substances), defence from conspecifics, and interestingly, provisioning of pre-digested carrion to the larvae. In some species parental care is obligate (Capodeanu-Nägler et al. 2016), while in those where it is facultative, parental provisioning typically improves larval fitness despite larvae being able to self-feed (Eggert and Muller 1997; Smiseth and Moore 2002; Smiseth et al. 2003) However, the carcass is often a limited resource, setting the stage for conflict and cooperation within families, between parents, parents and offspring, and between siblings (Egert and

Muller 1997; Scott 1998). Combined with flexibility in the extent of parental care (both within and between species), this makes *Nicrophorus* beetles particularly valuable for understanding how ecological conditions influence family dynamics (Eggert and Muller 1997).

Nicrophorus vespilloides is the perhaps the most well studied burying beetle to date (Capodeanu-Nägler et al. 2016). Like other *Nicrophorus* species, *N. vespilloides* larvae can self feed from the carcass, but have better growth and survival when provisioned by parents (Smiseth et al. 2003). They can be bred and maintained easily under laboratory conditions for generations; hence, there have been a plethora of focused experimental studies investigating family dynamics in this species and how they are influenced by a range of factors, including variation in resource availability (see for example Smiseth and Moore 2002). Despite this extensive literature on the *N. vespillodes* social system, there have not yet been any comprehensive investigations of sibling competition over parental provisioning in the context of Hamilton's rule.

Birds

In most animals, offspring from a given reproductive bout hatch, emerge or are born within a relatively short time of each other, relative to the time required for their development (Stoleson and Beissinger 1995). In other words, hatching – or birthing where live-bearing occurs – is synchronous. In contrast, the eggs of most bird species hatch over an extended time span (hatching asynchrony), usually from hours to days (Magrath 1990). At a proximate level, hatching asynchrony results initially from the fact that birds are constrained to lay eggs one at a time. Then, because the development of chicks in eggs – and the time until they hatch – is dependent on the onset of incubation by parents, parents can control patterns of hatching asynchrony through their incubation behaviour.

There is much variation in hatching patterns between taxa, particularly at the taxonomic levels of family and order (reviewed in Stoleson and Beissinger 1995). As such, variation in hatching patterns within this taxon are likely to represent at least some level of true adaptation rather than simply reflecting phylogenetic constraints. Some view hatching asynchrony as result of selection on incubation behaviour rather than the patterns they produce; for example, there may be selection for parents to

reduce the time that eggs and chicks are in the nest and vulnerable to predation (Dunlop 1910). Therefore, they will begin incubation on the first egg, creating a hatching pattern that has no adaptive value in and of itself. Others view the patterns that are created by hatching asynchrony to be adaptive themselves, with females altering early incubation functioning to produce those patterns. Typically, these patterns result in offspring size and dominance hierarchies; last hatched offspring are smaller, less dominant and suffer reduced growth and/or higher mortality compared to their older siblings (Lack 1947; Bryant 1978; Mock et al. 1990). This results from the reduced ability of the youngest offspring to obtain food during scramble competition or fights with their siblings over parental provisioning (Mock et al. 1990). The differential costs and benefits of hatching asynchrony for parents versus offspring and between siblings makes it an interesting trait in the context of the evolutionary ecology of parental reproductive strategies and within family conflict. Specifically, it has been suggested that in environments with fluctuating and often limited resources, hatching asynchrony is a parental strategy that maximises their overall fitness, but at a cost to their last-hatched offspring; the optimum clutch size for parents is the maximum clutch size that can be reared under high resource conditions, but in the case that resources are scarce, they still ensure some fitness returns by prioritising a core group of offspring and sacrificing the last hatched offspring (either actively, or passively by allowing the first hatched offspring to outcompete the last). This long-standing hypothesis (first proposed by Lack 1947) has received much empirical attention, but with little consensus as to whether it has any general explanatory power for the evolution of hatching asynchrony (reviewed in Stoleson and Beissinger 1995). In my thesis I use meta and comparative analyses across bird species to examine the fitness consequences of hatching asynchrony for parents versus offspring and between siblings and explore the dependency of these consequences on ecological conditions, thereby providing a general and quantitative test of this hypothesis.

Thesis structure and presentation

The thesis contains five chapters in total; four experimental chapters, and a chapter that uses meta-analytical and comparative approaches. In the first of the experimental chapters **(chapter two)** I quantified and explored the drivers of variation in levels of conflict between socially monogamous pairs of *Liopholis whitii*, the most important

family dyad that provides the social environment for family living to occur once offspring are produced. Next **(chapter three)** I quantified the levels of conflict between different dyads in *L. whitii* family units; specifically, I quantified and compared levels of mother-offspring, father-offspring and sibling conflict. Here I found that both sibling and father-offspring conflict were high. As previous work (While et al. 2009a) already showed that genetic relatedness drives father-offspring conflict in this system, in **chapter four** I tested how genetic relatedness between siblings and experimental manipulation of food availability affects sibling conflict. In this chapter I also aimed to link behavioural conflict to concomitant effects on family structure, by testing how these factors influence offspring dispersal. In **chapter five**, I continued exploring the drivers of sibling conflict in a different species that displays simple and facultative family living, *N. vespillodies*. Here I used a fully factorial experiment manipulating relatedness between siblings and the resources available to them to examine how these factors influence sibling competition over access to parental provisioning. Finally, in **chapter 6** I performed a series of meta-analyses and a comparative analysis of hatching asynchrony in birds, testing the idea that this parental reproductive strategy has differing fitness consequences for different family members; specifically, that parental fitness is enhanced under certain resource conditions, but at a cost to last hatched offspring, thus representing a mechanism that adjusts within family conflicts.

All these chapters except for chapters four and six are currently published. As such, each chapter is written as a stand-alone piece of work and may incur some repetition. This is particularly true for the chapters focussing on *L. whitii*, where there is some repetition in terms of methods describing the species' biology, collection and husbandry of study animals, and experimental protocols. As the chapters presented have been modified from manuscripts prepared for submission, each chapter may also vary slightly in formatting due to the specific requirements of each journal, particularly regarding referencing and the presentation of figures and tables. I take intellectual ownership for overall thesis presentation and contents, as reflected in primary authorship of all chapters.

I finish with a general discussion that synthesises the results of the studies carried out during my thesis. Here I also integrate these results into a conceptual framework that

highlights potentially useful broad research approaches for understanding of the evolution of complex kin-based sociality.

To explore and develop other ideas related to my thesis aims, I carried out an additional study during my candidature that tested for the presence of maternal effects in *L. whitii* (published paper provided as appendix one). In addition, the large-scale integrated nature of the *L. whitii* system resulted in collaboration on a publication that does not relate directly to the content of my thesis. Specifically, this paper experimentally tested the effects of food availability to mothers during gestation on the cognitive abilities of offspring. This work was published during my candidature at the University of Tasmania and so is also included in my thesis as an appendix (published paper provided as appendix two).

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CHAPTER TWO

Experimental manipulation suggests effect of polyandry but not mate familiarity on within-pair aggression in the social skink, *Liopholis whitii*

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Abstract

Long-term social monogamy is a key characteristic of family living across animals. The evolutionary maintenance of long-term monogamy has been suggested to be facilitated by increased reproductive coordination as a result of mate familiarity, leading to increased reproductive success. However, such effects can be compromised if females mate outside the pair bond (e.g. female polyandry), introducing conflicts of interest between the male and female. Here we experimentally test the effects of both mate familiarity and female polyandry on agonistic behaviour and reproduction in a family living lizard, *Liopholis whitii*. We found that mate familiarity did not decrease the level of aggression between pairs. In contrast, we found that manipulating polyandry did increase aggression between pair members. However, this did not have concomitant effects on female reproductive output. These results suggest that male behavioural responses to female promiscuity will influence the stability of pair bonding in these lizards, providing support for the growing appreciation of the multiple ways in which female polyandry can influence the stability of family living.

Significance statement

Family living is underpinned by social pair bonds between adults (i.e. stable social monogamy). Therefore, key to understanding the emergence and maintenance of family living is identifying factors influencing these bonds. We manipulated both female polyandry and mate familiarly in a replicated enclosure experiment using social lizards to test their role in influencing within-pair aggression and ultimately the coordination of reproductive behaviour and hence reproductive output. We found that polyandry but not familiarity influenced levels of aggression between pairs, but this did not transmit into concomitant effects on reproductive output.

Introduction

Family living is characterised by the presence of long-term pair bonds between adults and prolonged adult-offspring associations. The evolutionary maintenance of long-term pair bonds (hereafter social monogamy) has been suggested to be favoured because partners that have been together for an extended period of time may be more coordinated in their reproductive behaviour, resulting in increased reproductive output (the "mate familiarity" hypothesis – Black 1996). However, while there is empirical evidence that reproductive investment increases with the length of the pair bond (e.g. Black 2001; Pyle et al. 2001; van de Pol et al. 2006; Adkins-Regan and Tomaszycki 2007; Griggio and Hoi 2011; Sánchez-Macouzet et al. 2014), the majority of studies have been unable to separate out the effects of pair stability from that of male and female breeding age (van de Pol et al. 2006; Sánchez-Macouzet et al. 2014). To address this, we need studies which can manipulate pair familiarity in an experimental context and examine the consequences for pair coordination and ultimately reproductive success.

The benefits of social monogamy will not only depend on the length of the pair bond, but also on a number of other social behaviors. One behavior that can disrupt pair bond stability is when females pursue and accept copulations from males outside the pair bond (Taylor et al. 2014). Indeed, female polyandry has been shown to increase intersexual aggression between males and females (Valera et al. 2003), increase the risk on infaticide by the social male (e.g. Robertson 1990; Osorio-Beristain and Drummond 2001) and reduce paternal investment in care, including male desertion (e.g. Griffin et al. 2013). In the long term, persistent female polyandry can result in the evolutionary dissolution of social monogamy and the emergence of a more promiscuous social and mating systems (Kokko 1999).

Here we experimentally examined the effects of pair familiarity and female polyandry on male-female behaviour and female reproductive output in a family living lizard, *Liopholis whitii*. *Liopholis whitii* belong to the *Egernia* group of family living lizards characterised by large diversity in both their social and mating behaviour, from solitary species through to those that form large communal family groups (reviewed by Chapple 2003; Gardner et al. 2015; While et al. 2015). Importantly, social organisation across the group is underpinned by long term pair bonds and relatively low levels of extra-pair

paternity (Chapple 2003). Indeed, in some species, pairs have been recorded to last more than 25 years (e.g. in the sleepy lizard *Tiliqua rugosa*; Leu et al. 2015). One explanation for the maintenance of long term monogamy in this and other species in the *Egernia* group is that it results in increased coordination of reproductive behaviour and ultimately enhanced reproductive success (Bull 2000; Leu et al. 2015). Furthermore, female polyandry may influence the stability of family living within this system through its effects on within-family conflict (While et al. 2009a). To test these hypotheses, we experimentally manipulated pair familiarity and female polyandry in a fully factorial design and examined the extent to which this influenced (a) levels of aggression between male and female partners and (b) the consequences of this for female reproductive output.

Methods

Study species

White's skink (*Liopholis whitii*) is a medium-sized (up to 100 mm snout vent length, SVL) viviparous skink distributed throughout a wide altitudinal range (0-1600 m) and broad habitat types in south-eastern Australia (Cogger 2014; Chapple 2003; Wilson and Swan 2013). We used *L. whitii* from a population on the east coast of Tasmania, Australia (42°57'S, 157°88'E). Individuals at this study site are found in discrete patches of open grassland in close proximity to excavated burrows or rock crevices that are used as retreat sites. *Liopholis whitii* reproduce annually, with mating occurring during the austral spring (September – October) (While et al. 2009b). Gestation spans 3-4 months and birth of offspring occurs in the austral summer (January – February). Tasmanian populations of *L. whitii* live in stable social groups typically consisting of a single female and her male partner, often along with a cohort of 1-3 juvenile or sub-adult individuals (While et al. 2009b). Approximately 70 % of adults exhibit stable long-term pair bonds (While et al. 2009b), with pair bonds lasting up to 12 – 15 years (GMW et al. unpublished data). Levels of extra-pair paternity are moderate within this population, with extra-pair offspring comprising about 30% of the offspring born each year (While et al. 2009b).

Field and experimental methods

We captured a total of 120 adult *L. whitii* (72 males and 48 females) at the start of the breeding season (early September) in 2015. Lizards were captured using mealworm fishing and noosing

techniques (as outlined in While et al. 2014). At their time of capture, individuals were weighed (± 1 mg), measured (snout-vent length, total length ± 0.5 mm) and toe clipped for permanent identification. Toes were kept for DNA analysis to allow later assignment of paternity (see below). Lizards were then released into small (1 m diameter) outdoor enclosures at the University of Tasmania's animal compound. Each enclosure was supplied with a brick block for basking, a 30 x 15 cm steel sheet for shelter, along with water and food (*Tenebrio* larvae) provided *ad libitum.* Each of 48 enclosures housed a male-female pair. The remaining 24 un-partnered males were also housed in these enclosures, but individually and used as extra-pair males for the polyandrous treatment (see below).

We manipulated pair familiarity and female polyandry in a 2 by 2 factorial design. To manipulate pair familiarity, we constructed male female pairs from either lizards that had been caught in the same burrow system (n = 24) or by constructing male-female pairs from lizards that had been caught in separate burrows (n = 24). Shared burrow use by a single male and his female partner is the key characteristic of *L. whitii* pairs, which rely on these permanent burrow sites to undertake the majority of their basking, foraging and social behaviours (While et al. 2009a; While et al. 2011; While et al. 2014; see also Chapple 2003; Chapple and Keogh 2006). We crossed our manipulation of pair familiarity with a manipulation of female polyandry, by creating monogamous and polygynous treatment groups. To achieve this we gave females access to either only their social partner or their social partner and additional males during a 3-week mating period (mating period in *L. whitii* goes from mid-September to mid-October; McEvoy et al. 2013). Specifically, for the monogamous treatment, females were given access to only their social partner for the duration of the trial. In contrast, females in the polyandrous treatment had their social male partner removed and replaced with an extra-pair male (from one of the 24 un-partnered males). The extra-pair male was with the female for two days of the week before being removed and replaced with the female's original male partner. The females in the monogamous treatment also had their social male partner removed during the same period (as a control), but without a male replacing him during his absence. Three male removal and return cycles occurred over 3 weeks, up until the completion of the mating season (mid-October). Each female in the polyandrous treatment had access to two extra pair males in total (one male in weeks 1 and 2, and a second, different male in week 3). The extra pair males chosen for each female were selected based on size (such that size differences in SVL were minimised)

and genetic structure; only extra pair males caught between 40 and 200 m from a given female were mated, to avoid any female mating biases based on inbreeding or outbreeding effects (see While et al. 2014; Bordogna et al. in press).

To quantify levels of male-female aggression between pair members, we recorded pair interactions between each male and female pair once a day for a four-week period following the mating season using GoPro cameras (Hero3+, California, USA). One-hour periods were filmed of the female and her social partner. Filming occurred between 0900 and 1200, when temperatures are most suitable for high levels of lizard activity. From the footage we could record three key variables associated with aggression and conflict between adults: chasing, biting and fleeing (see While et al. 2009a; McEvoy et al. 2013 for a detailed description of aggression in these lizards). Biting describes one individual biting their partner, chasing describes the action of one individual aggressively chasing their partner without contact being made, and fleeing describes the action of an individual attempting to escape an enclosure by scrambling in the enclosure's periphery (independently of being chased by their partner). All behavioural observations were collected by two observers (TBJ and JS) and videos were scored blind with regard to treatment to minimise observer bias. A subset (n = 12) of videos were scored by both observers to confirm inter-observer reliability, which was found to be high in all cases (Cohen's kappa (k) greater than 0.75 for each variable; $k_{\text{biting}} = 1.00$, $k_{\text{chasing}} = 1.00$, $k_{\text{fleeing}} = 0.79$; Kaufman and Rosenthal 2009).

At the end of female gestation (mid-January), individuals were moved into the indoor terrestrial ecology facilities at UTAS, where they were housed individually in plastic terraria as described above. Female containers were checked at 2 h intervals daily for the birth of offspring. For each offspring, the date of birth, weight (± 1 mg), SVL and total length (± 0.5 mm) were recorded. Offspring were toe clipped for permanent identification, with toes kept to allow DNA analysis for later assignment of paternity (see below). Male and female pairs were then released along with their offspring at their site of capture.

Parentage assignment and confirmation of polyandry manipulations success

All individuals included in the study were genotyped for three microsatellite loci (EST1, EST2, EST4: Gardner et al. 1999) using standard molecular techniques with DNA extracted from tail tip samples (see While et al. 2011 for further details). We only used 3

microsatellites because a) the low number of potential dads meant a limited number of microsatellites were required to distinguish between potential fathers and b) the aim of parentage assignment was simply to confirm that our manipulation of polyandry resulted in mating between the female and the extra pair male. Paternity was assigned using the computer program CERVUS 3.0 (Marshall et al. 1998) using the following simulation parameters: 10 000 cycles, 95% of candidate parents sampled, 95% loci typed, and a genotyping error rate of 1% (calculated in CERVUS from our data). The "one known parent" option was used, with all adult males released into the same enclosure as the mother included as possible fathers. Paternity was assigned to the male with the highest male–female–offspring trio LOD score and the lowest number of mismatches (0 or 1) (e.g. Gardner 2002; Chapple and Keogh 2005; While et al. 2011).

Data analyses

Data were analysed using a combination of ANOVAs, Generalized Linear Models (GLMs), and Generalized Linear Mixed Models (GLMMs) fit by maximum likelihood. These were run in R version 3.1.0 (R development core team 2015) using the 'aov' function for ANOVA models, the 'glm' function for the GLM, and the 'glmer' function for GLMMs (Bates et al. 2016). For GLMMs, Laplace approximation was used to estimate model parameters, as it is a more accurate technique than the simpler and widely used pseudo quasi-likelihood estimation method (Bolker et al. 2009). Estimates of fixed effects for all models were obtained with the 'car' package (Fox et al. 2016). We report results for models containing all main effects and significant interactions following backward elimination of non- significant interactions. Results are reported as means, with standard errors as the measure of variability. All data were checked for violation of assumptions, and no violations were found.

We examined differences in the level of aggression between males and females within their pairs as a function of the two treatments using GLMMS with a poisson distribution. Specifically, three models were run separately with frequency of biting, chasing and fleeing behaviours as dependent variables. Pair familiarity and polyandry treatment were entered as fixed factors and time was included as a covariate (to account for some pairs being filmed for slightly longer overall than others). Models were initially overdispersed, but this was corrected by including a subject level random effect to account for overdispersion. For 27 videos we could identify the male and female. For

these videos we ran three additional GLMMS (with a poisson distribution) to examine differences between males and females in the frequency of biting, chasing and fleeing behaviours. Additionally, changes in female weight were analysed (non-pregnant females were used, to avoid confounding weight changes with clutch mass) from the start to the finish of the mating season to assess whether our treatments had any consequences for females in terms of reduced body condition. This was analysed using a type III ANOVA.

To examine the consequences of the two treatments, and the subsequent differences in conflict between the two treatments on female reproductive traits we ran several models. First, we used a GLM with the binomial family specified to test for any differences between the treatments in successfully producing offspring. Secondly, we analysed whether there were any differences between treatments in female reproductive output (relative clutch mass; Shine 1980), average birthdate of offspring, average offspring mass, and average offspring condition). Offspring condition measurements were calculated by dividing mass by SVL (Green 2001). These analyses were conducted using type III ANOVAs. Each model included litter size as a covariate.

Results

In total, 500 hours of adult interaction footage were recorded, giving us 2,302 independent observations of aggression and avoidance behaviours between individuals. There was no effect of mate familiarity on the frequency of fleeing, biting or chasing within pairs. However, males and females chased each other and retreated more in the polyandrous treatment compared to the monogamous treatment. (Table 1, Figure 1). Males were the main instigators of aggressive interactions in almost all instances. Indeed, males were observed chasing (4.66 ± 1.07 chases/hour) and biting (3.59 ± 0.60 bites/hour) females significantly more often than females were observed chasing (0.81 ± 0.81 chases/hour) and biting (1.4 ± 0.79 bites/hour) males (chases, $\chi^2 = 80.74$, $p < 0.001$; bites, $\chi^2 = 25.56$, $p < 0.001$). There was no difference in the number of times males and females were observed trying to flee (7.92 ± 2.95 flees/hour vs 7.18 ± 3.42 flees/hour; $\chi^2 = 0.98$, $p = 0.32$). There was no significant effect of treatment on female weight change from the start to the end of the mating period (Table 1).

Fourteen out of the 42 females (33%) recaptured at the end of the mating period gave birth, resulting in a total of 35 offspring. Paternity analysis confirmed that our polyandrous

treatment resulted in a successful manipulation of extra pair paternity, with 6 out of 7 clutches (86%) produced by polyandrous females containing at least one extra-pair offspring. There was no difference in the likelihood of giving birth between either treatment (Table 2). We also found no significant difference between either familiar and unfamiliar pairs or monogamous and polyandrous pairs in birth date, relative clutch mass, average offspring mass or average offspring condition (Table 2).

Table 1 Outputs of analyses of treatment effects on conflict between pairs and female weight change, after removal of non-significant interactions. Significant effects are bolded

Response variable	Familiar vs. unfamiliar	Monogamy vs. polyandry	Time
Frequency of bites	$\chi2_{(1)} = 1.51$, p = 0.22	$\chi2_{(1)} = 2.42$, p = 0.12	**$\chi2_{(1)} = 4.37$, p<0.04**
Frequency of chases	$\chi2_{(1)} = 0.07$, p = 0.79	**$\chi2_{(1)} = 15.11$, p<0.01**	$\chi2_{(1)} = 2.11$, p = 0.15
Frequency of fleeing	$\chi2_{(1)} = 0.41$, p = 0.52	**$\chi2_{(1)} = 4.93$, p<0.03**	**$\chi2_{(1)} = 8.00$, p<0.01**
Female weight change	$F_{(1,24)} = 0.60$, p = 0.45	$F_{(1,24)} = 0.60$, p = 0.45	

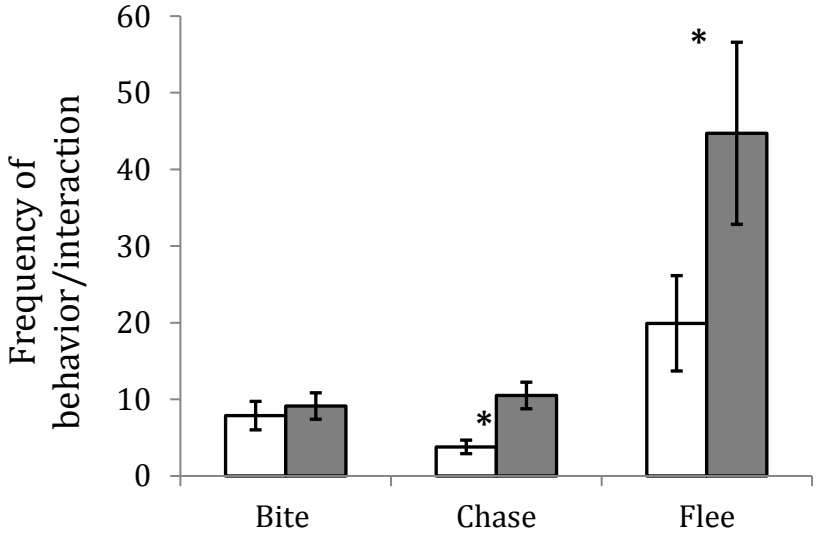

Figure 1: Differences between polyandrous (grey fill) and monogamous (white fill) pairs in the frequency of biting, chasing and fleeing. Asterisks indicate where there were significant differences. Error bars represent one standard error of the mean.

Table 2 Outputs of analyses of treatment effects on traits measuring female reproductive output, after removal of non-significant interactions. Significant effects are bolded.

Response variable	Familiar vs. unfamiliar	Monogamy vs. polyandry	Litter size
Reproduced (Y/N)	$Z_{(1,45)} = 0.63$, p = 0.53	$Z_{(1,45)} = 0.00$, p = 1.00	
Relative clutch mass	$F_{(1,11)} = 0.15$, p = 0.70	$F_{(1,11)} = 0.80$, p = 0.39	
Birth date	$F_{(1,10)} = 0.0.73$, p = 0.41	$F_{(1,10)} = 0.01$, p = 0.94	$F_{(1,10)} = 0.93$, p = 0.36
Average offspring mass	$F_{(1,10)} = 4.22$, p = 0.07	$F_{(1,10)} = 1.00$, p = 0.34	$F_{(1,10)} = 0.02$, p = 0.89
Average offspring condition	$F_{(1,10)} = 3.62$, p = 0.09	$F_{(1,10)} = 0.42$, p = 0.53	$F_{(1,10)} = 0.02$, p = 0.91

Discussion

There is a considerable interest in understanding the evolution and maintenance of stable social monogamy (reviewed in Reichard and Boesch 2003). One of the key explanations for the maintenance of stable monogamous pair bonds is that it enhances reproductive performance via increased reproductive coordination (Black 1996). Our study represents the first examination of pair dynamics and reproductive output in response to experimental manipulation of mate familiarity in a reptile (and, to the best of our knowledge, a non-avian species more generally). Furthermore, we combined this manipulation with a manipulation of polyandry, which can create conflicts of interest between the female and her social partner, leading to a disruption of stable social monogamy and its benefits. We showed that polyandry, but not mate familiarity, resulted in a significant exaggeration of agonistic behaviours between males and females within a pair, indicative of increased within-pair conflict. In contrast, we found no effect of either treatment on female reproductive output, but our statistical power to detect smaller effect sizes was limited. Below, we discuss potential explanations for these results and their broader implications for understanding the evolution of social monogamy.

One of the primary explanations for the maintenance of long term pair bonding, including in *Egernia* lizards, is that mate familiarity enhances reproductive output through enhanced pair coordination (Bull 2000; See also Black 1996). For example, recent research on long term pair bonding in *Tiliqua rugosa* showed that long term pairs mated earlier in the mating season than shorter term pairs, which may enhance offspring survival if earlier mating correlates with earlier offspring birth date (Bull 1988; Leu et al. 2015). However, we found little evidence that mate familiarity influences pair aggression/coordination in *L. whitii*, nor did we not find

evidence that it increases reproductive output. These results may reflect the nature of the mating system in *L. whitii*. Specifically, in contrast to the majority of other systems used to explore the mate familiarty hypothesis (e.g. Black 2001; Pyle et al. 2001; van de Pol et al. 2006; Adkins-Regan and Tomaszycki 2007; Griggio and Hoi 2011; Sánchez-Macouzet et al. 2014), including *T. rugosa* (Leu et al. 2015), where pairs separate outside the breeding season but return to the same partner the following year, *L. whitii* live with their social partner for the duration of the breeding and non-breeding period (Chapple and Keogh 2006; While et al. 2009b). As a result, pair familiarity may be of less functional significance for the finding, re-acquainting and priming of social partners in *L. whitii* compared to other systems. However, it is important to note that the moderate number of females who went through reproduction (33%, on average 60% of female *L. whitii* go through reproduction in a year in the wild; While et al. 2009a; b) within our experimental set up limited our ability to tease apart more subtle differences in reproductive investment between familiar and unfamiliar partners. However, irrespective of this, the lack of differences in aggressive interactions and the lack of a difference in the likelihood of giving birth between familiar and unfamiliar pairs still suggests limited support for the mate familiarity hypothesis.

In contrast to mate familiarity, we did find a significant effect of polyandry on intra-pair aggression. Specifically, pairs in the polyandrous exhibited increased aggression towards one another compared to pairs in the monogamous treatment, despite that male removal occurred in both treatments. Furthermore, aggression was primarily directed toward the female by the male. This suggests that males are be able to assess the risk of polyandry directly via chemical recognition mechanisms, as has been shown for other species (e.g. the sand lizard *Lacerta agilis*; Olsson et al. 2004). This provides further evidence that kin recognition functions in a wide number of contexts in the *Egernia* (e.g. mate choice; While et al. 2014; parental care, While et al. 2009a). The increased aggression associated with mating outside the pair bond is consistent with theoretical suggestions which suggest that aggression may serve as a male adaptation to punish females for undertaking extra-pair copulations (Johnstone and Keller 2000). However, despite substantial work on extra-pair paternity this study represents one of the few empirical examples that the inferred risk of paternity increases aggression between pair members (see also Valera et al. 2003).

Increased aggression as a result of polyandry could have significant implications for female fitness given that increased inter-sexual aggression has been shown to influence reproductive

output in female lizards (Le Galliard et al. 2005). However, we did not find any direct consequences of enhanced conflict between polyandrous pairs for female reproductive output. Whether the increased inter-sexual aggression resulting from polyandry has more subtle effects on female fitness requires additional work. For example, Le Galliard et al. (2005) found no effect of inter-sexual aggression on female reproductive output in the following reproductive event, they did find a negative effect of inter-sexual aggression of female reproductive output when measured across a female's lifetime. Irrespective of the extent of direct effects of polyandry on female fitness, our results here suggest that extra pair mating by females may undermine the stability of pair bonds in this system through increased conflict. This supports previous research suggesting a negative effect of female polyandry on family stability in this system, via its effects on paternal investment in offspring tolerance (While et al. 2009a).

In conclusion, we have shown through an experimental approach that polyandry but not mate familiarity influences within-pair aggression in a socially monogamous lizard. This adds to the growing body of work articulating the extent to which female mating behaviour can have fundamental implications for the maintenance and diversification of complexity sociality (e.g. Cornwallis et al. 2010; Griffin et al. 2013). Moving forward, more work is required to understand the factors responsible for the origins and maintenance of long-term pair bonds in this system. We suggest that limited resource availability (mates and/or territories) may be important. *Egernia* lizard ecology is characterised by high habitat saturation, with strong territoriality and relatively long lifespans creating low breeder turn-over and intense competition over access to limited permanent crevice sites (O'Connor and Shine 2004; Langkilde et al. 2005; While et al. 2009b). This may elevate the risk of being left without a mate or territory when switching mates between breeding seasons (see also Choudhury 1995) resulting in selection on the maintenance of pair bonds between seasons. Data on the costs and benefits of long-term pairing in the wild and well-replicated experimental manipulations of habitat availability across seasons may help further our understanding of the role resources play in stable pair bonding (e.g. Halliwell et al. 2016).

Compliance with ethical standards

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All work was carried out with approval from the Animal Ethics Committee at the University of Tasmania (Ethics Approval Number A0015058).

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CHAPTER THREE

Family aggression in a social lizard

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Abstract

The evolution of family living is underpinned by conflict and cooperation between family members. While family groups can be maintained by reducing conflict between parents and offspring, interactions between siblings may play an equally important role. Here, we compared the level of aggressive interactions between siblings to that between parents and their offspring in family groups of the social skink *Liopholis whitii*. Aggressive interactions occurred much more frequently between siblings and between fathers and offspring than between mothers and their offspring. These results suggest that ecological and social conditions that reduce conflict between siblings and between males and offspring are key factors for the evolutionary maintenance and diversification of family living in these lizards.

Introduction

The evolution of family living is mediated by within group conflict. Research has largely focused on parent-offspring conflict and the factors that affect the costs and benefits of prolonged parental investment for both parents and offspring [1,2]. However, conflict between siblings may be just as important, or more so, than conflict between parents and offspring for mediating family life [3-5]. Indeed, conditions that reduce conflict or facilitate cooperation between siblings can stabilise the social system, allowing the emergence of larger family groups [6]. Conversely, conditions that increase conflict between siblings can result in the dissolution of family living [7,8]. Yet, the relative roles of parent-offspring vs sibling-sibling conflict for the initial origins of family life are not well understood.

Here we compared the levels of parent-offspring and sibling-sibling aggression, as a proxy for conflict, in the social skink *Liopholis whitii*. *Liopholis whitii* live in small family groups characterised by a long-term adult pair bond and prolonged parent-offspring associations [9-11]. These prolonged associations involve offspring delaying dispersal and parents tolerating offspring within their core home ranges, sometimes for up to several years. These parent-offspring associations extend to semi-independent offspring remaining within the parental home range gaining access to resources and protection from conspecifics [12]. Importantly, parent-offspring associations are facultative and there is considerable variation in their strength; from no association between parents and offspring to associations between parents and multiple cohorts of offspring [13,14]. Furthermore, there appears to be no costs of associating with offspring for parents [12], whereas offspring pay a considerable cost of associating with their siblings via increased competition for food and shelter [7,8]. This suggests that aggression between siblings should be high compared to parent-offspring aggression, and that variation in the extent of sibling conflict may explain variation in social complexity in this species. Here, we tested the first of these predictions, i.e., that aggression between siblings should be high compared to parent-offspring aggression.

Methods

Collection and housing of study animals

Liopholis whitii is a medium sized (75-100 mm snout-vent length (SVL)) viviparous skink that occurs throughout south-eastern Australia [15]. We captured 103 adult lizards (95 females and 8 males) for three separate experiments in 2014, 2015 and 2016. In all cases, lizards were captured in the field and transported in cool, damp cloth bags back to the University of Tasmania. At the University, lizards were weighed (± 1 mg), measured for SVL and total length (± 0.5 mm) and sexed via hemipene eversion. Each lizard was uniquely toe-clipped to enable individual identification. Lizards were then housed individually in plastic terraria (30 x 60 x 40 cm) kept under a 25 W basking light set to an 8:16 hour light/dark cycle with overhead lights set on a 10:14 hour light/dark cycle. Each terrarium had a basking rock underneath the basking light, with a wooden shelter at the opposite end. Lizards were provided with water daily and food three times a week (*Tenebrio* larvae and fruit puree mixed with protein powder).

At the end of gestation (mid-Jan), female containers were checked at 2 hour intervals for the birth of offspring. Upon birth, the date of birth, weight (± 1mg), SVL and total length (± 0.5mm) of offspring were recorded and toe clipped for permanent identification. A coloured 'bee tag' was attached at a point along the dorsal side on the individual, using non-toxic glue (Pender Beekeeping Supplies) to allow for instant identification of individuals on camera footage (see below).

Interaction trials

We recorded interactions between different family dyads (sibling-sibling, mother-offspring, and father-offspring) by filming family groups following birth. On the day following the birth of all her offspring (determined via abdominal palpation), females and their offspring were filmed in their terraria for a one hour period using a GoPro Hero4 camera (California, U.S.A). In the 2016, experiment we also recorded father-offspring conflict on the day following filming of offspring with their mother, using the same protocol. All interaction trials started at 0900 hrs (following the 1hr acclimation). Following each interaction trial, animals were either released back into their natural population or into semi-natural enclosures as part of on-going projects. Across the three experiments we recorded mother-offspring interactions from 95 one hour videos and sibling-sibling interactions from 89 one hour videos. From the 2016 experiment we recorded father-offspring interactions from 8 one hour videos.

Ethics statement

All experimental protocols were carried out in accordance with the Australian code of practice for the care and use of animals for scientific purposes as approved by the University of Tasmania Animal Ethics Committee (project numbers: A15058, A14380 and A14602).

Data analysis

From the video footage we recorded the number of times individuals bit and chased one another as our measure of aggressive behaviour. These two variables were highly correlated (spearman's rank correlation = 0.79, $p<0.01$). We report results using frequency of chases as our measure of aggression instead of frequency of bites, as 1) chases were easier to quantify based on video footage and therefore provided a more definitive response variable and 2) chasing is a more active process than biting and hence more representative of aggression for this species. However, when running analyses with frequency of bites as our conflict measure results remained significant and our interpretations were identical.

Differences in levels of sibling and parent-offspring aggression were modelled using a Generalized Linear Mixed Model (GLMM) fit by maximum likelihood. This was run in R version 3.3.0 (R development core team 2016) using the 'glmmADMB' package [16] with the negative binomial family specified to account for overdispersion. Family dyad (sibling-sibling, mother-offspring, or father-offspring) was entered as a fixed effect, clutch size was entered as a covariate, and experiment year (2014, 2015, or 2016) and litter identity were included as non-nested random effects (litter identity was included to control for non-independence arising from having individual litters provide measurements of both sibling and parent-offspring conflict). Post-hoc comparisons were undertaken using the Tukey's HSD method for p-value adjustment (implemented through the 'lsmeans' package [17]). We did not sample males in the 2014 and 2015 experiments and for the 2016 experiment there were extremely low levels of mixed paternity (only two litters; Botterill-James et al. unpubl data). Thus, we could not conduct any formal tests of the role that relatedness may play in mediating conflict (see discussion).

Results and Discussion

There was a significant difference between the family dyads in the extent of aggressive behaviour (Wald's $\chi^2_{(2)} = 24.33$, $p < 0.01$). Chasing between siblings occurred in 57.3 % of interaction trials, fathers chased offspring in 75% of trials whereas mothers only chased offspring in 3.2% of trials. Post hoc analyses revealed that both sibling-sibling and father-offspring aggression were significantly higher than mother-offspring conflict (z ratio = 8.06, $p < 0.01$ and z ratio = 4.41, $p < 0.01$ respectively; Figure 1) but did not differ significantly from each other (z ratio = -0.64, $p = 0.79$; Figure 1).

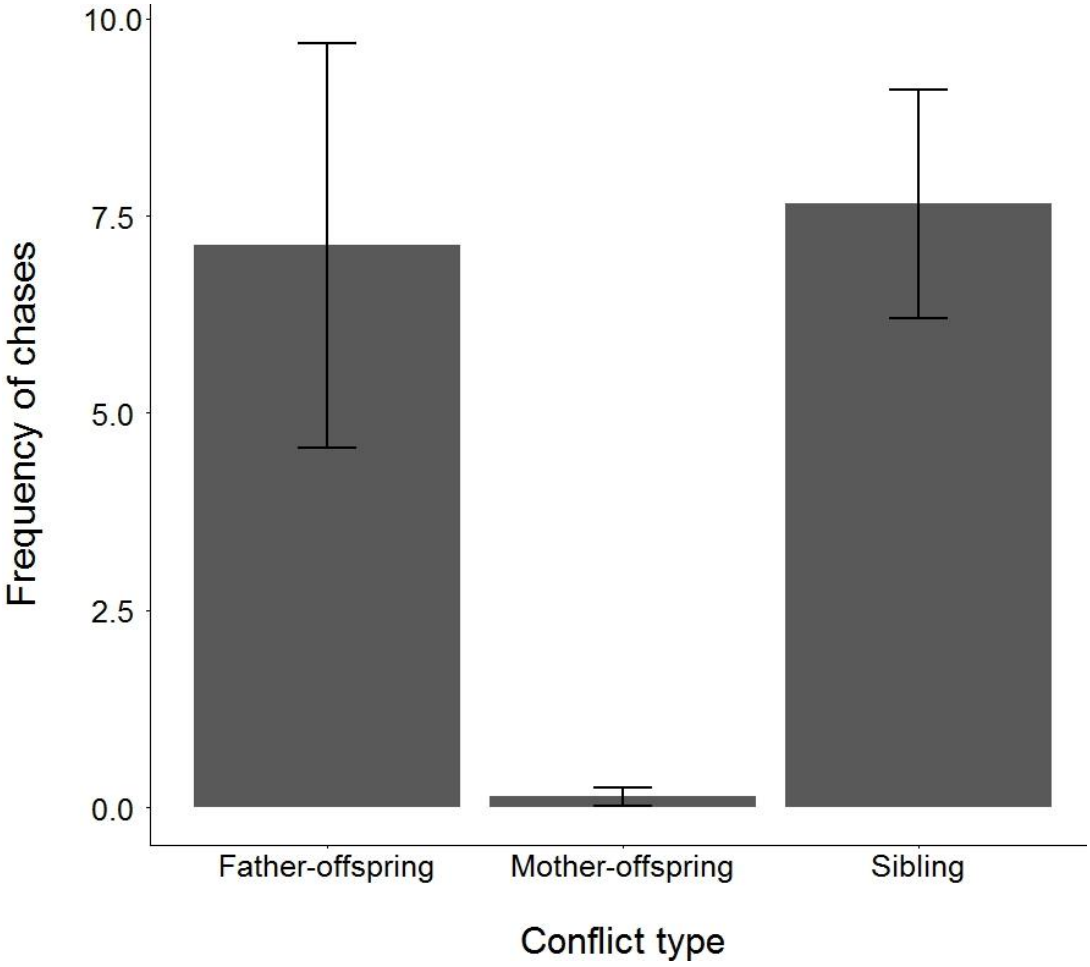

Figure 1: The level of aggressive behaviour, measured as chasing behaviour, between mothers and their offspring, fathers and their offspring and between siblings within *Liopholis whitii* families. Error bars represent one standard error of the mean.

These results are consistent with other family living species, particularly birds and mammals, where sibling aggression has significant consequences for offspring growth, dispersal and survival [18]. Our results suggest that aggressive interactions between siblings may play an important role in mediating the composition of the family groups in *Liopholis whitii.* Perhaps the most likely consequence of sibling aggression is that it influences the extent to which individuals disperse out of the natal home range and the identity of dispersers. However, sibling aggression is unlikely be the sole mediator of family dynamics. We found that aggressive interactions between fathers and their offspring occurred just as frequently as between siblings. Therefore, male tolerance of offspring within their natal home range is also likely to be important. In contrast, we found little evidence of mothers exhibiting aggression towards their offspring. This is probably because, in contrast to many birds and mammals, *Liopholis whitii* do not exhibit post-hatching provisioning and the cost of tolerating offspring is low [12]. Nevertheless, mothers may shape sibling conflict because birth of offspring is often spread out across several days, which influences the outcome of aggressive interactions between siblings [19]. Hatching asynchrony is important in this respect in birds and deserves further attention in *Egernia* lizards.

These results suggest the maintenance of family living in White's skink, and perhaps the evolutionary diversity of social organisation in the *Egernia* lizards [13], will primarily be dictated by factors that mediate sibling and paternal-offspring conflicts. Such within-group conflicts are mediated by patterns of relatedness between individuals and the costs and benefits of tolerating other individuals [20]. Within-group relatedness will largely be dictated by female polyandry whereas the costs and benefits of tolerating other individuals will largely be a function of the environment, in particular resource availability and predation risk. Both these factors are likely to be important in *L. whitii.* First, we have previously shown considerable levels of polyandry (~30%) within natural populations of *L.whitii* and that this influences the composition of the family group by promoting enhanced dispersal of extra-pair offspring [11]. Second, experimental manipulations of resource availability have been shown to mediate offspring dispersal and the level and nature of parent-offspring associations [21]. The behavioural mechanisms mediating these facultative responses have not been directly studied, but our results here may indicate that social interactions with kin are involved. Studies that

manipulate levels of female polyandry and examine the consequences for within group conflict could tease apart the causal relationship between polyandry, within group conflict and the evolutionary diversification of social complexity as inferred by recent comparative analyses [1,6].

In summary, our results suggest that the stability of lizard family life relies on reducing conflict between siblings and between fathers and offspring [1,3]. Since these conflicts depend on both female polyandry and ecological opportunity, studies that manipulate these factors will provide insights into the maintenance and diversification of family living both within and between lizard species.

Authors' contributions

All authors conceived of and designed the study. TBJ, BH, JS and SM caught field animals and recorded video footage. TBJ recorded interaction data from the video footage and conducted statistical analyses with input from GW and BH. TBJ, GW and TU drafted the manuscript with input from all other authors. All authors gave approval for publication and agree to be held accountable for the content therein.

Competing interests

The authors declare no competing interests

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CHAPTER FOUR

No effect of polyandry or food availability on sibling conflict in a family living lizard

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Abstract

Conflict is a persistent challenge to the stability of family life. Family conflicts are predicted to be reduced when both levels of relatedness between family members (the *r* term in Hamilton's rule) is high and competition over resources (a proxy for the *B* and *c* terms of Hamilton's rule) is low. Despite this, empirical studies of within family conflict tend to only focus on one of these aspects at a time. Furthermore, such studies typically do so in the context of parent-offspring interactions in species that exhibit complex and obligate sociality. Here, we examined the role of both relatedness and the resource availability in mediating sibling conflict in a reptile that exhibits a simple and facultative form of family living, *Liopholis whitii*. We did this in a hierarchical experiment where we first measured the frequency of sibling conflicts (measured as chases between siblings) in response to (1) experimental manipulation of food availability during different stages of offspring development, and (2) variation in sibling relatedness resulting from natural variation in female mating behaviour (monogamous vs. polyandrous mating) using lizard families (mothers with their offspring) housed together in small enclosures. We then tested whether these same factors affected broader patterns of offspring dispersal and family structure using large semi-natural populations in a mesocosm experiment. We found that neither polyandry nor food availability had a consistent effect across our two experiments. We discuss these results in the context of similar studies in other systems, and implications for understanding the early evolution of family living.

Introduction

The maintenance of kin groups is mediated by reduction of within-group conflicts. Research in this context has largely focused on parent-offspring conflict and specifically the factors that affect the costs and benefits of prolonged parental investment for both parents and offspring (Clutton-Brock 1991; Royle et al. 2012). However, interactions between siblings may be just as important, or more so, than interactions between parents and offspring for social group stability (Falk et al. 2014; Ruch et al. 2014a, b; Kramer and Meunier 2018). Indeed, such interactions have been explored in the context of the complex family and cooperative breeding systems found in many bird and some mammal species (e.g., Mock and Parker 1997; Dickinson and McGowan 2005; Baglione et al. 2006; Boncoraglio and Saino 2008; Raihani et al. 2008; Boncoraglio et al. 2009; Hodge 2009; Klemme and Ala-Honkola 2014; Caro et al. 2016; Koenig and Dickinson 2016) and eusocial insects (Giron et al. 2004; Segoli et al. 2009a, b; Polidori and Borruso 2012; Schultner et al. 2014). However, such interactions are also likely to be important to the stabilisation of social interactions during the early stages of social evolution, that is, for the simple and facultative family groups that represent the precursors to more complex social systems (Falk et al. 2014; Ruch et al. 2014a, b; Kramer et al. 2015; Schrader et al. 2015; Botterill-James et al. 2017a; Kramer and Meunier 2018). Therefore, exploring factors that influence the extent of conflict vs. cooperation between siblings in simple and facultative family groups is crucial for understanding their evolution, and through this, the evolution of complex sociality more generally.

The extent of conflict between siblings should depend on (1) the level of relatedness between siblings, and (2) the relative costs and benefits of conflict (Hamilton 1964). Therefore, any factors that influence these traits should be important in mediating the maintenance of family living. Two factors are likely to play a key role in this context. First, low levels of polyandry (female mating with multiple males) should increase relatedness between siblings, thereby reducing conflict/increasing cooperation (Hamilton 1964; West et al. 2007). Such effects, both in facultative and broader macro-evolutionary studies, has been demonstrated in a range of taxa, including birds (Briskie et al. 1994; Mock and Parker 1997; Baglione et al. 2006; Boncoraglio and Saino 2008; Boncoraglio et al. 2009; Cornwallis et al. 2010; Caro et al. 2016), mammals (Hodge 2009; Lukas and Clutton-Brock 2012; Klemme and Ala-Honkola 2014) and sub-social (Falk et al. 2014; Ruch et al. 2014a, b) and eusocial insects (Giron and Strand 2004; Giron et al. 2004; Segoli et al. 2009a, b; Hughes et al. 2008; Boomsma et al. 2011;

Schultner et al. 2014). Second, for a given level of relatedness, food availability should be important in mediating the costs and benefits of conflict behaviours. Specifically, high food availability should decrease both the costs of tolerating siblings, thereby reducing conflict/increasing cooperation (Mock et al, 1987; Drummond 2001; West et al. 2002; Bourke 2014), which has been demonstrated across a range of taxa; especially in birds and mammals (reviewed in Mock and Parker 1997, see also e.g., Machmer and Ydenberg 1998; Golla et al. 1999; Cook et al. 2000; Nathan et al. 2001; Osorno and Drummond 2003; Hodge 2009; White et al. 2010). Despite this, few studies have tested (1) the effects of relatedness and food availability simultaneously on family interactions, and (2) done so in the context of sibling interactions in simple and facultative family groups that represent the precursors to more complex sociality.

While these factors can have direct effects on levels of sibling conflict, they can also be mediated indirectly by parents. Indeed, parents are expected to actively mediate conflict within their brood whenever they have the potential to benefit, positively or negatively, from the extent and outcome of sibling conflict. For example, where sibling conflict allows for simple brood reduction in times of unpredictable resource limitation, it can have positive effects on parental fitness (Lack 1947; Mock and Parker 1997). However, sibling conflict can also be detrimental to parental reproductive success if it decreases overall brood performance and recruitment (Drummond 1993: Roulin and Dreiss 2012). Parents can mediate conflict in a brood in a variety of different ways. This can be achieved either directly, for example, by punishing aggressive offspring (Cash and Evans 1986; White 2008) or it can be done in more nuanced ways. For example, parents can mediate sibling conflict through differential allocation of resources (such as hormones) to offspring before birth (Muller et al. 2007; Smiseth et al. 2011) or by spacing the birth of offspring by birthing/hatching asynchrony (Hahn 1981; Mock and Parker 1997; Smiseth et al. 2007).

Here we tested the effects of polyandry and food availability on sibling conflict in the facultatively family living skink, *Liopholis whitii*. *Liopholis whitii* live in small family groups characterised by a long-term adult pair bond and prolonged associations between parents and offspring (Chapple and Keogh 2006; While et al. 2009b). These prolonged associations involve offspring delaying dispersal and parents tolerating offspring within their core home ranges, sometimes for up to several years (Chapple and Keogh 2006; While et al. 2009b). The prolonged association with parents provides

several benefits to offspring, including access to food, basking sites and protection from infanticidal conspecifics (Botterill-James et al. 2016). Importantly, these parent-offspring associations are facultative, resulting in considerable variation in family structure; most often involving no association between parents and offspring or association between one-two offspring and their parents, and more rarely, associations between parents and multiple cohorts of offspring (Chapple and Keogh 2006; While et al. 2009a, b). Family life is characterised by high conflict between siblings (Botterill-James et al. 2017b), and *L. whitii* individuals are able to discriminate between kin (While et al. 2009b; While et al. 2014) potentially via olfaction (Bull et al. 2000). This suggests that sibling conflict could be a key mechanism determining variation in family structure observed in *L. whitii* populations, by influencing the trade-off between offspring philopatry and dispersal (Botterill-James et al. 2017b; Halliwell et al. 2017a). Furthermore, such sibling conflict should vary in relation to with both relatedness (as a kin recognition mechanism is present) and food availability. In addition, *L. whitii* exhibit asynchronous birth of offspring, with the extent of birthing asynchrony variable between mothers and giving rise to sibling size hierarchies (While et al. 2007); birthing asynchrony may potentially be an indirect way in which mothers may influence sibling conflict. We therefore tested (1) how polyandry and food availability affect sibling conflict (using an experiment with small outdoor enclosures); (2) how these factors influence offspring dispersal and thereby family composition (using a mesocosm experiment); and (3) whether sibling conflict is controlled by mothers via birthing asynchrony.

Methods

We utilised an integrated approach to address to the above aims. Specifically, we first used a small-scale enclosure experiment, where each enclosure housed a single *L. whitii* mother (and her offspring after birth – see below), to test the effects of food availability (experimentally manipulated) and polyandry (natural variation) on sibling conflict. We then used a mesocosm experiment, with enclosures fitted with offspring dispersal gates and each enclosure housing a small 'population' of *L. whitii* families. This allowed us to test the effects of these same factors on offspring dispersal and family dynamics at the population level.

Small-scale enclosure experiment

Study species and study animals

White's skink (*Liopholis whitii*) is a medium-sized (up to 100 mm snout vent length, SVL) viviparous (live-bearing) skink distributed throughout a wide altitudinal range (0-1600 m) and broad habitat types in south-eastern Australia (Chapple 2003). We used *L. whitii* from a population on the east coast of Tasmania, Australia (42°57' S, 157°88' E). Individuals at this study site are found in discrete patches of open grassland in close proximity to excavated burrows or rock crevices that are used as retreat sites. *Liopholis whitii* reproduce annually, with mating occurring during the austral spring (September – October) (While et al. 2009b). Gestation spans 3-4 months and birth occurs in the austral summer (January – February). Litters comprise of one to four offspring, and most frequently is two (While et al. 2007). Approximately 35% of these litters contain extra-pair offspring (While et al. 2009b).

Our small-scale enclosure experiment here is also described in detail in Botterill-James et al. (2019). We captured a total of 70 pregnant *L. whitii* at the end of the breeding season (early November) in 2016 using mealworm fishing and noosing techniques (details in While et al. 2014). Pregnant females were caught after the end of the breeding season, and soon after ovulation (Cartledge and Jones 2007). This meant that females spent most of gestation under experimental conditions. These pregnant females were then brought to the terrestrial ecology facilities at the University of Tasmania, where they were weighed (± 1 mg), measured for length (snout-vent length (SVL)), total length ± 0.5 mm) and toe clipped for permanent identification. They were then housed individually in plastic terraria (30 x 60 x 40 cm) and kept under a 25 W basking light set to an 8:16 hour light/dark cycle, with overhead UV basking lights set on a 10:14 hour light/dark cycle. Each terrarium had a basking rock underneath the basking light, with a wooden shelter at the opposite end of the terrarium. Animals were held under these conditions for a maximum of 7 days before random allocation into their experimental treatments (see below).

Experimental design, food manipulation and data collection

Our experimental design crossed high and low food availability treatments during gestation (hereafter GH and GL respectively) with high and low food availability treatments post-birth (hereafter PH and PL respectively). For the duration of both treatments the lizards were individually held in small outdoor enclosures at the University of Tasmania. These enclosures were 1 m in diameter and consisted of a Bessa block for basking, a 30 x 15 cm piece of sheet metal for shelter, along with water provided *ad libitum.* All enclosures were covered with bird netting to prevent predation. Enclosures were identical in terms of the vegetation within them, spaced closely together, and treatments were randomly assigned to enclosures, thereby minimising any confounding environmental effects.

Females were randomly assigned to each treatment. For the gestation treatments, females in the GH treatment received five regular mealworms (size range of individual worms = 0.16 – 0.24 g) three times weekly during gestation (November until birth, which occurred from early February onwards), while females in the GL treatment received one regular mealworm three times weekly. All females in both treatments typically consumed all their food and did so within an hour (TBJ pers. obs.). Examination of female body condition (calculated as scaled residuals from a least squares regression of body mass against length: Peig and Green 2010) just after birth showed that our treatments were successful in manipulating the amount of energy available during gestation. Specifically, females under the high food treatment during gestation had a 9.17% increase in condition, while females under the low food treatment had a -0.07% decrease in condition (type III SS ANOVA: $F_{(1,66)} = 74.29$, $P < 0.01$).

At the end of female gestation (mid-January), individuals were moved into indoor terrestrial ecology facilities until they gave birth (for most indivudals, approximately a month from when they were moved indoors), where they were housed individually in plastic terraria as described above. Female containers were checked at 2 h intervals daily for the birth of offspring, to enable us to record the extent of birthing asynchrony for each clutch. For each offspring, the date of birth, weight (± 1 mg), SVL and total length (± 0.5 mm) were recorded. The average date of birth of a mother's first offspring was the same in both treatments (21/2/2017, GH range = 5/2/2017 to 16/3/2017, GL range = 3/2/2017 to 20/3/2017). As all offspring retain hemipenes at birth, we were

unable to determine the sex of offspring, and thus offspring sex was not considered further. Following the collection of morphometric data, offspring were toe clipped for permanent identification. After the birth of each offspring, the mother was palpated gently to check if any offspring remained to be born. Once all offspring were born, the clutch was released back into the outdoor enclosure with their mother and each family was randomly assigned to a post-birth food availability treatment.

The post-birth high and low food availability treatments were identical to those prior to birth. However, to account for the presence of offspring along with their mother, we doubled the weight of food available. Specifically, instead of receiving either five or one mealworms three times weekly, the enclosures received twice the equivalent weight in 'mini-mealworms' (size range of individual worms = 0.04 – 0.06 g). Mini-mealworms were substituted for regular sized mealworms as observations in the laboratory suggest offspring can choke while attempting to eat regular sized mealworms. Mothers and offspring were kept under these conditions for a six-week period after which they were returned to the laboratory where they were measured for final weight and SVL, and any mortality of offspring was recorded. As with our manipulation of the pre-birth treatment, data on offspring and mother condition between the two treatments suggested that our manipulation of food availability influenced food intake (see Botterill-James et al. 2019 for details). Additionally, we checked if any effects of the post-birth environment on offspring were mediated the behaviour of the mother in this period – i.e., mothers who received low food during gestation or post birth could consume more food in the post-birth environment, therefore creating parent-offspring competition that might mask or exacerbate sibling conflict (e.g., Schrader et al. 2015). To check this, we analysed whether the gestation treatment affected mothers' change in condition from birth until the end of the experiment, but found no effect (type III SS ANOVA: $F_{(1,37)} = 0.71$, $P = 0.41$). Further, there was no effect of a mother's postnatal change in weight on the change in SVL or condition of her offspring ($F_{(1,28.29)} = 0.17$, $p = 0.68$), suggesting no or limited parent-offspring competition in our experiment.

Estimating relatedness between siblings

Mothers and offspring were genotyped for 12 microsatellite loci (EST1, EST2, EST4, ECU2, EST12, TRL28, ECU5, TRL12, TRL1, ECU1, TRL21, TRL34: Gardner et al. 1999;

2008) using standard molecular techniques with DNA extracted from tail tip samples (see While et al. 2011 for further details), to enable us to calculate sibling relatedness and classify each clutch as being of single or mixed paternity. Relatedness (sensu Queller and Goodnight 1989) was calculated using the software package COANCESTRY (Wang 2011); clutches with any siblings having a relatedness of ~0.25 were classified as being of mixed paternity (n =15), and clutches with all offspring having a relatedness of ~0.5 to each other were classified as being single paternity (n = 20). One clutch of two offspring had a relatedness between the siblings of 0.38, so it was ambiguous as to whether the clutch was of single paternity origin; we classified the clutch as single paternity, but as our results did not change qualitatively when rerunning our models with this clutch classified as mixed paternity, we report our results here with this clutch classified as single paternity. The clutches of the remaining 35 females were not able to be classified as single or mixed paternity, because either the female failed to produce any offspring or only produced one offspring (n = 24), one offspring died soon after birth and so no conflict data was recorded (n = 4), or DNA was unable to be successfully extracted from the tail sample of one of the offspring in a clutch of two (n = 7).

Recording conflict between siblings

Conflict data for each clutch was collected at two time points; (1) 24 hours after the birth of all offspring (to examine the effect of the gestation food treatment and relatedness on conflict), and (2) after three weeks of exposure to the post birth food treatment (to examine the effect of the post-birth food treatment and relatedness on conflict). All video recordings started at 0900 hrs and filming was conducted using a GoPro Hero4 camera (California, U.S.A). For all videos, we scored interaction data from the second hour of footage (following 1 hr of acclimation); specifically, we recorded the number of times individuals chased one another as our measure of conflict (see Botterill-James et al. 2017b for further details of the recording procedure and use of chasing as a conflict proxy).

Data analysis

Data were analysed using Generalized Linear Mixed Models (GLMMs) in R (version 3.5.2, 2018). We examined (1) the effects of the gestation food treatment and sibling relatedness (and its interaction) on sibling conflict recorded at birth, (2) the effects of

the post birth food treatment and sibling relatedness (and its interaction) on sibling conflict recorded after three weeks spent in the outdoor enclosures, and (3) the effect of birth spread on sibling conflict recorded at birth. These models were all run with the Poisson family specified and included clutch size as a covariate and an observation level random effect to account for overdispersion found in the original models (each model was first run as a Generalized Linear Model). We also examined at an individual level whether the amount of conflict received (as recorded at birth) predicted offspring mortality. We ran this analysis using a GLMM with the binomial family specified and maternal ID included as a random effect. The significance of fixed effects are reported based on Wald chi-square tests. For all these analyses we report results for models containing all main effects and significant interactions following backward elimination of non-significant interaction terms.

Mesocosm experiment

Study animals

Different lizards from a nearby site were used in a separate mesocosm experiment. We captured a total of 30 pregnant *L. whitii* towards the end of gestation (early February) and 12 males (from a nearby third site) in 2017 using mealworm fishing and noosing techniques. These pregnant females were then brought to the terrestrial ecology facilities at the University of Tasmania, where they were weighed (± 1 mg), measured for length (snout-vent length (SVL), total length ± 0.5 mm) and toe clipped for permanent identification. They were then housed individually in plastic terraria (as described for the small-scale enclosure experiment), and for each offspring, the date of birth, weight (± 1 mg), SVL and total length (± 0.5 mm) were recorded. Animals were held under these conditions for a maximum of seven days before random allocation into one of two experimental treatments (see below) and release into large outdoor enclosures at the University of Tasmania's Cambridge Farm facility.

Mesocosm experimental design and setup

The mesocosm experiment consisted of two treatments, (1) a low food availability treatment and (2) a high food availability treatment, replicated three times each. Each experimental replicate was comprised of 2 adjacent 8 × 8 m enclosures (i.e. an

enclosure pair) with identical food and separated by a partition, creating one "home" and one "dispersal" enclosure (see Figure S1). We installed 3 dispersal gates made of 8-mm mesh in the partition separating each enclosure pair, allowing offspring to move between enclosures within a pair but prohibiting the movement of adults (see Halliwell et al. 2017a). Food was manipulated for enclosure pairs in a similar manner as described for the small enclosure experiment. Specifically, in the low food treatment, no food was provided (other than naturally occurring invertebrates), whereas enclosure pairs in the high food treatment were supplemented with ~50 grams of meal worms distributed throughout the enclosure pair every two days. Five wooden pallets, each covered with ~100 L of burrowing substrate and topped with cement bricks facing each major aspect, were spaced evenly throughout each enclosure, creating five high quality crevice sites similar to habitats used by *L. whitii* in natural populations (see Botterill-James et al. 2016; Halliwell et al. 2017a, b).

We released five lizard families (mum and her offspring) into each "home" enclosure, giving a total of 30 mothers and 59 offspring included in the experiment. This meant that home enclosures were entirely saturated, with one habitat patch for each female and her offspring. Finally, we released two males into each "dispersal" enclosure. Adult males are highly aggressive to unrelated offspring (O'Connor and Shine 2004; While et al. 2009a), so by releasing males into dispersal enclosures we introduced a dispersal cost to prevent universal offspring dispersal occuring, which would prevent analysing variation in patterns offspring dispersal across treatments.

Before release, we attached numbered cloth stickers (Tesa, Germany) to the back of each adult female and a coloured bee tag fixed with nontoxic glue (Pender Beekeeping Supplies, Australia) to each offspring for identification. The bee tags were colour coded with five different colours corresponding to each of the five mothers within a home enclosure. We used the position of a tag along an offspring's back to differentiate which member of a clutch it was (i.e., tag fixed to neck = 1st born offspring, middle of the back = 2nd born, pelvis = 3rd born) and therefore its unique ID. Mothers and their offspring were randomly allocated to treatments before release, resulting in 28 and 31 offspring in the high and low food treatments respectively.

Quantifying offspring dispersal

We collected positional data and behavioural interactions on all visible individuals twice a day in a morning and afternoon observation session. This allowed us to calculate estimates of offspring dispersal behaviour and instances of mother-offspring associations (see below, Halliwell et al. 2017a for details). In each of these sessions we spent 20 min observing each enclosure pair, recording which enclosure each individual was in ("home" or "dispersal") and their physical position within the enclosure. Afternoon observation sessions commenced at least four hours after the completion of morning sessions to reduce spatial autocorrelation. At the end of the experiment we recaptured all mothers and offspring and returned them to the laboratory to measure size, mass and body condition before releasing them back to their initial capture location.

During the two-month observational period, we recorded 1055 positional observations. The average number of observations for mothers, offspring and males during our experiment was 9.8 ± 1.3, 10.4 ± 1.1, and 12.2 ± 1.4 respectively. For mothers, we used observational point data to calculate 50% kernel contours via least-squares-cross-validation (LSCV) in the computer program Ranges 9 (Anatrack Ltd, UK). We set a constant LSCV smoothing factor of 0.75 when generating kernels as this was most effective at buffering against over-smoothing and under-smoothing of kernels at the extremes of the sample size range (see Kie 2013). We then used the 50% kernel of each female to identify which crevice site she had utilized as her primary retreat site. After identifying sites occupied by each female, we defined juveniles as delaying dispersal and associating with their mother if 50% or more of their observations were on or within 1 m of that crevice site. This corresponds with the distribution of offspring locations around female crevice sites in the field (While et al. 2009a, b; GM While unpublished data) and has been successfully implemented in previous mesocosm studies of offspring delayed dispersal and philopatry in this species (Botterill-James et al. 2016; Halliwell et al. 2017a). For females with 50% kernels including more than one crevice site, observations of juveniles falling within 1 m of any of these sites were included.

Determining exploration, settlement, and delayed dispersal

We defined two nonexclusive classes of dispersing individuals, coded as binomial variables, for the purpose of statistical analyses. Explorers were offspring who were

observed within the dispersal enclosure at least once during the experiment. Settlers were offspring observed in the dispersal enclosure in at least three sequential observations and never observed back in the home enclosure. Previous work on the effects of habitat saturation on dispersal showed that three sequential observations is sufficient to identify settlers, correctly identifying 95% of settlers (Halliwell et al. 2017a). We excluded offspring with fewer than three observations (n = 16) from all analyses except those taking "explored" and "survived" as response variables as the reliability of these responses did not depend on total observation number; a single observation of an individual in the dispersal enclosure is proof of exploration and the recapture of an individual at the end of the experiment is proof of survival.

In contrast to this settlement category, delayed dispersal implies a persistent association between parents and offspring within the natal range. Therefore, offspring were only considered to have delayed dispersal if they overlapped their mother's core home range area (see above). Importantly, although offspring classified as explorers could either go on to settle or delay dispersal, these were not the only possible behavioral responses; offspring could establish a home range within the home enclosure that did not overlap that of their mother, resulting in a negative response for both these categories. Furthermore, settle and delay dispersal were treated as mutually exclusive behaviours; any offspring classified as having settled could not be considered to have also delayed dispersal. This classification was never contentious, as offspring who settled never had ≥50% of their observations within the maternal home range.

Estimating relatedness between siblings

We estimated relatedness between siblings using the methods described for the small enclosure experiment and using genetic samples of offspring taken at birth. We had nine single paternity clutches and five mixed paternity clutches. The clutches of the remaining 16 females were not able to be classified as single or mixed paternity, because the female produced only produced one offspring (n =7), or because or DNA was unable to be successfully extracted from the tail sample of one of the offspring in a clutch of two (n = 9).

Data analysis

As for the small enclosure experiment, all data from the mesocosm experiment was analysed in R (v 3.5.2, 2018). We first examined whether the food treatment affected offspring change in snout-vent length and mass, to ensure that our food manipulation successfully translated into altered food availability. For both these models, we used linear mixed models (LMMs), with change in SVL or change in mass as response variables, food availability treatment as a fixed effect, enclosure density as a covariate, and mother ID nested within enclosure ID as a random effect. The significance of fixed effects are reported based on type III *F*-tests with degrees of freedom approximated by the Kenward–Rogers method. These models were checked for violation of assumptions (visual inspection of residuals plots for heteroscedasticity and non-normality of errors) with no violations being found.

Second, we examined whether the food treatment affected offspring survival using a generalized linear mixed model (GLMM) with the binomial family specified. Here, offspring survival (yes vs. no) was the response variable, with food treatment as a fixed effect, release SVL and enclosure density as covariates, and mother ID nested within enclosure ID as a random effect.

Third, to investigate the influence of the food availability treatment on offspring dispersal behaviour we fit three separate binomial GLMMs taking whether or not offspring explored, settled and delayed dispersal as response variables, treatment and paternity as fixed factors, enclosure density as a covariate (except in the delayed dispersal model due to model convergence issues) and mother ID nested within enclosure ID as a random effect. We then re-ran the three models as described above, but included paternity as a fixed effect, to test if it increased the predictive power of our models. We avoided fitting interactions for any of these offspring dispersal behaviour models as we had insufficient sample sizes to adequately test for interactions between fixed effects, but visually inspected plots to qualitatively check for any interactive effects between food availability and paternity. The effects of food availability on offspring dispersal behaviour reported in the results are from these models excluding paternity as a fixed effect. For all dispersal analyses, the significance of fixed effects is reported based on Wald chi-square tests.

Results

Effects of food availability and polyandry on sibling conflict; small-scale enclosure experiment

Of the 70 pregnant females we captured, 58 produced offspring (= 83% reproduced), resulting in 114 offspring. These levels of reproductive success and clutch sizes are similar to observed rates of reproduction in the wild (While et al. 2009b) and also of experiments carried out in captive populations previously (Botterill-James et al. 2016; Halliwell et al. 2017a, b).

The average birth spread for females for which we also had sibling conflict at birth data was 2.21 ± 0.22 days (range = 0 to 6.88 days, n = 42 litters) and did not differ between treatments ($F_{(1, 44)}$ = 0.26, p = 0.62). Increased birth spread decreased sibling conflict, but this effect was not significant (Wald's $\chi^2_{(1)}$ = 2.13, p = 0.15, table 1, fig 1).

Table 1: Parameter estimates with standard errors, test statistics and p-values for small enclosure experiment analysis of the effects of birthing asynchrony on sibling conflict at birth.

	Conflict		
	estimate	std. err	p
Intercept	Wald's chi-square = 0.93		
	-2.43	2.52	0.34
Birthing asynchrony	Wald's chi-square = 2.13		
(days)	-0.66	0.45	0.15
Clutch size	Wald's chi-square = 1.80		
	1.80	1.34	0.18

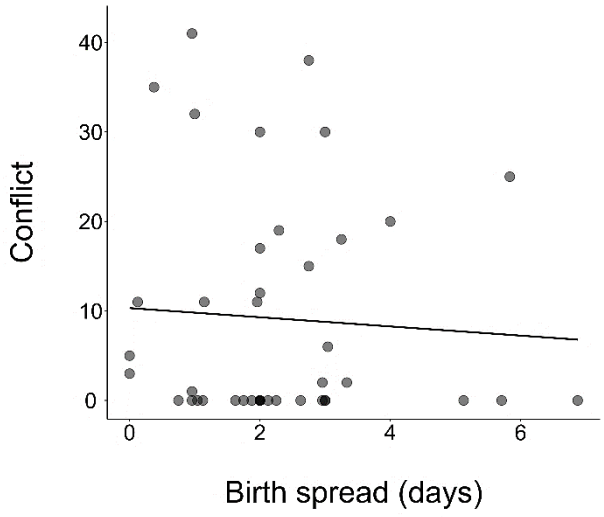

Figure 1: Conflict between *Liopholis whiii* siblings at birth (number of chases per interaction trial) in our small-enclosure experiment decreased non-significantly with birth spread. n = 42.

Conflict at birth was generally lower in single vs mixed paternity clutches (average number of chases in single paternity clutches = 7.95 ± 2.88, n =20, average number of chases in mixed paternity clutches = 12.40 ± 3.24, n =15). However, when analysed within a GLMM framework, neither sibling relatedness, the gestation food treatment, had a significant effect on conflict at birth (food availability, Wald's $\chi^2_{(1)} < 0.01$, p = 0.98, sibling relatedness, Wald's $\chi^2_{(1)} = 0.26$, p = 0.10, interaction, Wald's $\chi^2_{(1)} < 0.01$, p = 0.98, table 2, fig 2a).

Similarly, conflict between siblings at three weeks post-birth was generally lower in single than mixed paternity clutches (average number of chases in single paternity clutches = 6.73 ± 3.73, n =15, average number of chases in mixed paternity clutches = 13.47 ± 4.04, n =15) and in the low compared to the high food treatment (average number of chases in low treatment = 7.60 ± 2.59, n =15, average number of chases in high treatment = 12.60 ± 4.92, n =15). However, neither of these effects reached statistical significance (food availability, Wald's $\chi^2_{(1)} = 0.67$, p = 0.41, sibling relatedness, Wald's $\chi^2_{(1)} = 2.18$, p = 0.14, interaction, Wald's $\chi^2_{(1)} = 1.89$, p = 0.17, table 2 fig 2b).

Table 2: Parameter estimates with standard errors, test statistics and p-values for small enclosure experiment analyses of the effects of paternity and food availability on sibling conflict.

	Conflict at birth			**Conflict after three weeks**		
	estimate	std. err	p	estimate	std. err	p
Intercept	Wald's chi-square = 0.22			Wald's chi-square = 0.30		
	1.12	2.36	0.64	-1.29	2.36	0.58
Gestation treatment	Wald's chi-square < 0.01					
(low)	-0.02	1.09	0.98			
Post-birth treatment				Wald's chi-square = 0.67		
(low)				-0.78	0.95	0.41
Clutch paternity	Wald's chi-square = 2.64			Wald's chi-square = 2.18		
(single)	-1.77	1.09	0.10	-1.47	0.99	0.14
Interaction	Wald's chi-square < 0.01			Wald's chi-square = 1.89		
	-.07	2.31	0.98	-2.57	1.87	0.17
Clutch size	Wald's chi-square < 0.01					
	0.09	0.96	0.93			
Clutch size				Wald's chi-square = 1.80		
(at three weeks)				1.37	1.02	0.18

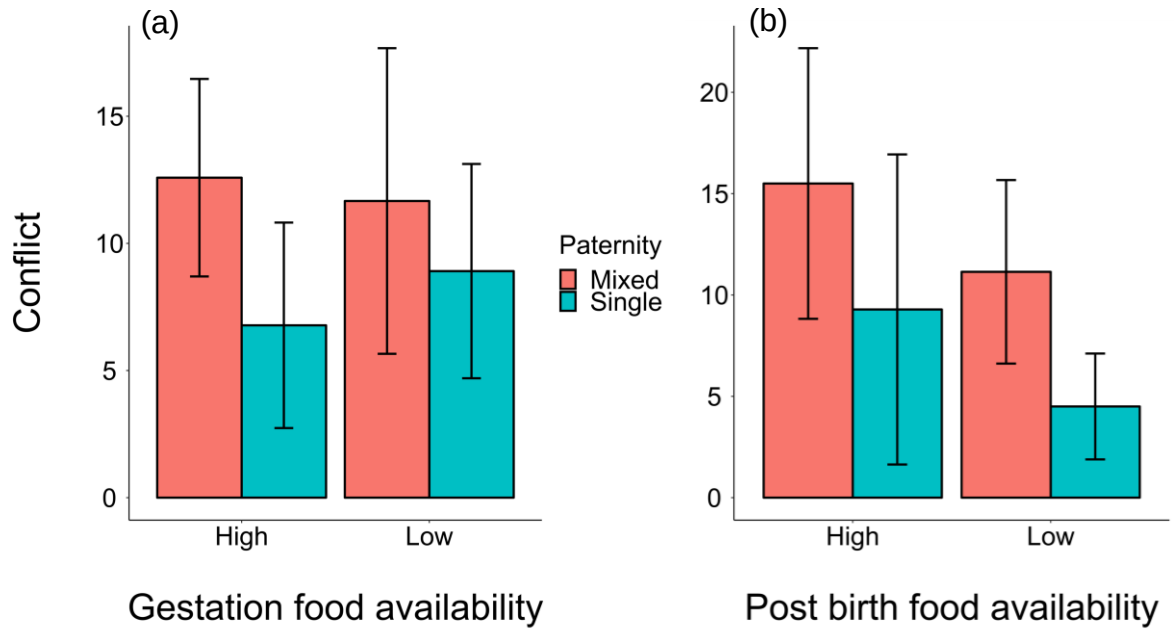

Figure 2: Conflict between *Liopholis whitti* siblings in response to paternity of clutches (mixed vs. single) and food availability in our small-scale enclosure experiment **(a)** Conflict at birth (number of chases per interaction trial) was not significantly affected by paternity of clutches or food availability during gestation. Sample sizes are; mixed paternity + high food, n = 12, single paternity + high food, n = 9, mixed paternity + low food, n = 3, single paternity + low food, n = 11. **(b)** Conflict after three weeks (number of chases per interaction trial) was not significantly affected by paternity of clutches or food availability during the post-birth period. Sample sizes are; mixed paternity + high food, n = 8, single paternity + high food, n = 7, mixed paternity + low food, n = 7, single paternity + low food, n = 8.

When examining the consequences of conflict for offspring survival, we found there was no effect of the amount of conflict an individual offspring received at birth on their risk of mortality (Wald's $\chi^2_{(1)} = 0.40$, $p = 0.53$). This result was qualitatively the same when using the amount of conflict received after three weeks as the predictor instead of conflict at birth (Wald's $\chi^2_{(1)} = 0.05$, $p = 0.82$).

Effects of food availability and polyandry on offspring dispersal behaviour; mesocosm experiment

We first examined whether the food treatment affected offspring growth (change in snout-vent length and mass) to assess if our food manipulation was successful in creating a high and low food availability treatment. Change in SVL was significantly greater in the high vs. low food treatment (8.44 ± 0.48 mm vs. 4.11 ± 0.58 mm, $F_{(1, 2.85)} = 28.57$, $p < 0.02$), as was mass (1.39 ± 0.10 g vs. 0.39 ± 0.08 g, $F_{(1, 3.23)} = 12.13$, $p < 0.04$). This confirmed that our treatment had the desired effect of altering energetic intake

available to lizards, as was the case for our small enclosure experiment (see Botterill-James et al. 2019; this study). We also examined whether the food treatment affected offspring survival and found a non-significant trend for greater survival among individuals in the high vs. low food treatment (76% vs. 54%, Wald's $\chi^2_{(1)} = 3.33$, p = 0.07).

In terms of the effect of food availability on offspring dispersal behaviour, we found that offspring were just as likely to explore in the high vs. low food treatment (11/26 = 42% of offspring explored in the high treatment, 13/28 = 46% of offspring explored in the low; Wald's $\chi^2_{(1)} = 0.01$, p = 0.91, table 3a, fig 3a). Similarly, offspring from single paternity clutches were just as likely to explore as those from mixed paternity clutches; 9/16 = 56% of offspring from single paternity clutches explored compared to 3/8 = 38% of offspring from mixed paternity clutches exploring (Wald's $\chi^2_{(1)} = 0.35$, p = 0.55, table 3b, fig 4b). There was no obvious (qualitative) interactive effect between food availability and paternity on offspring exploration behaviour (fig 3b).

Table 3a: Parameter estimates with standard errors, test statistics and p-values for mesocosm analyses of the effects of food availability on patterns of offspring dispersal. Significant effects are italicised.

	Explored			**Settled**			**Delayed dispersal**		
	estimate	std. err	p	estimate	std. err	p	estimate	std. err	p
Intercept	Wald's chi-square = 0.84			Wald's chi-square = 2.61			Wald's chi-square = 5.20		
	-1.79	1.94	0.36	-3.97	2.46	0.11	8.15	3.56	0.02
Food treatment	Wald's chi-square <0.01			Wald's chi-square = 1.88			Wald's chi-square = 6.89		
(low)	0.04	0.59	0.95	0,98	0.72	0.17	-18.11	6.90	<0.01
Enclosure density	Wald's chi-square = 0.60			Wald's chi-square = 1.34					
	0.15	0.20	0.44	0.28	0.24	0.25			

Table 3b: Parameter estimates with standard errors, test statistics and p-values for mesocosm analyses of the effects of paternity on patterns of offspring dispersal. Significant effects are italicised.

	Explored			**Settled**			**Delayed dispersal**		
	estimate	std. err	p	estimate	std. err	p	estimate	std. err	p
Intercept	Wald's chi-square = 0.27			Wald's chi-square = 1.07			Wald's chi-square = 1.15		
	-1.70	3.30	0.61	-5.39	5.21	0.30	2.58	2.41	0.28
Food treatment	Wald's chi-square <0.01			Wald's chi-square = 0.08			Wald's chi-square = 1.23		
(low)	-0.02	0.76	0.98	0.26	0.95	0.78	-3.40	3.07	0.27
Clutch paternity	Wald's chi-square = 0.35			Wald's chi-square <0.01			Wald's chi-square = 1.01		
(single)	0.83	0.49	0.55	0.01	1.14	0.99	-3.13	3.10	0.31
Enclosure density	Wald's chi-square = 0.10			Wald's chi-square = 0.66					
	0.10	0.32	0.75	0.42	0.51	0.42			

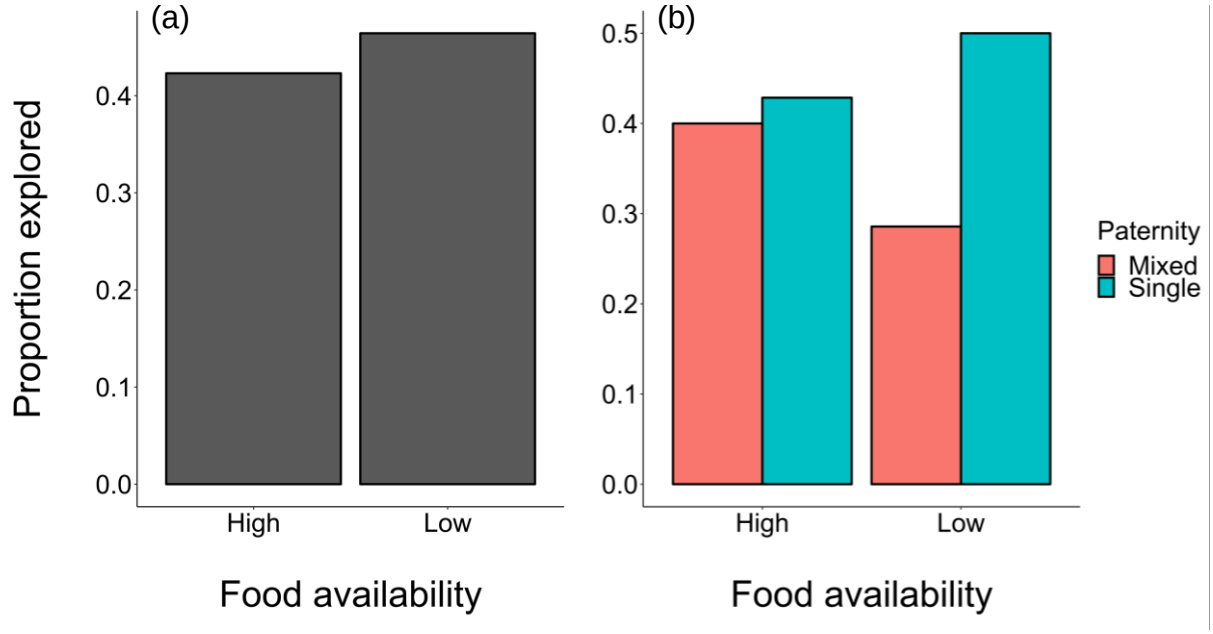

Figure 3: Exploration behaviour by *Liopholis whitii* offspring in our mesocosm experiment **(a)** Exploration by offspring was not significantly affected by the food treatment. Sample sizes are; high food availability, n = 26, low food availability, n = 28. **(b)** Exploration by offspring was not significantly affected by paternity of clutches (mixed vs. single) and there was no apparent interaction effect with the food availability treatment. Sample sizes are; mixed paternity + high food, n = 5, single paternity + high food, n = 6, mixed paternity + low food, n = 3, single paternity + low food, n = 10.

There was no significant effect of the food treatment on settlement, despite over twice the number of settlers in the low food treatment; 5/22 = 23% of offspring settled in the high treatment, 10/20 = 50% of offspring settled in the low (Wald's $\chi^2_{(1)} = 1.88$, p = 0.17, table 3a, fig 4a). Similarly, offspring from single paternity clutches were just as likely to settle as those from mixed paternity clutches; 6/16 = 38% of offspring from single paternity clutches settled compared to 2/8 = 25% of offspring from mixed paternity clutches settling (Wald's $\chi^2_{(1)} < 0.01$, p = 0.99, table 3b, fig 4b). There was no obvious interactive effect between food availability and paternity on offspring settlement (fig 4b).

Delayed dispersal was significantly greater in the high vs. low food treatment. Specifically, 11/17 = 65% of offspring delayed dispersal in the high food treatment, compared to 1/10 = 10% of offspring delaying dispersal in the low food treatment (Wald's $\chi^2_{(1)} = 6.89$, p <0.01, table 3a, fig 5a). There was no effect of paternity of clutches on offspring dispersal (Wald's $\chi^2_{(1)} = 1.01$, p = 0.31, table 3b, fig 5b) and no qualitative interactive effect between food availability and paternity (fig 5b)

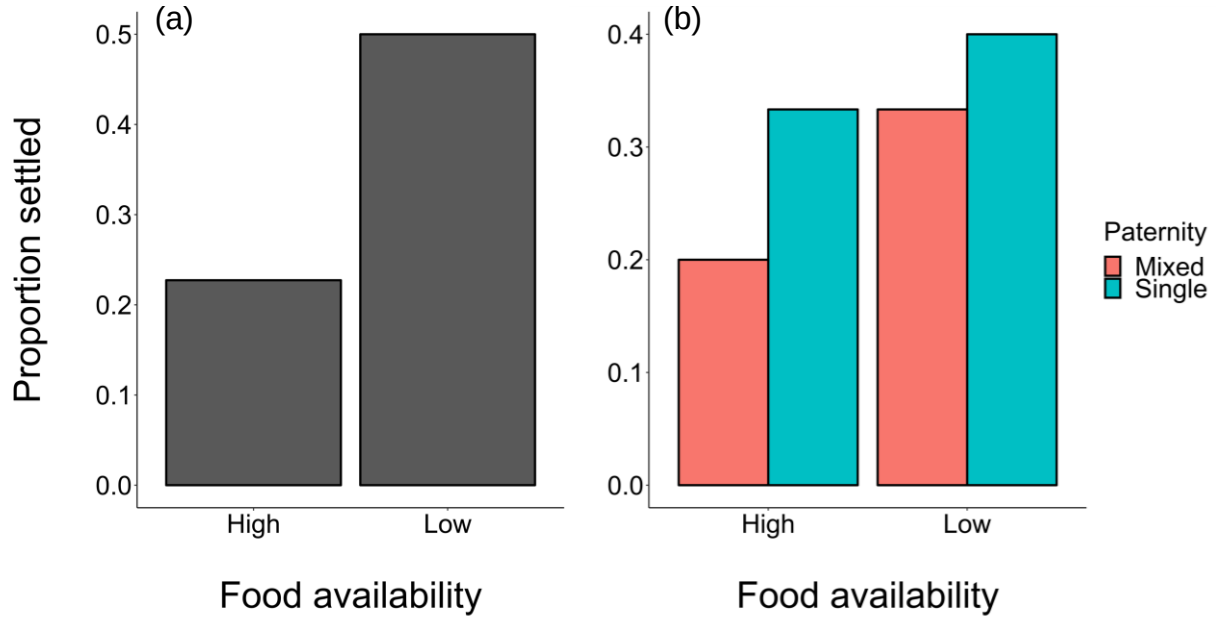

Figure 4: Settlement behaviour by *Liopholis whitii* offspring in our mesocosm experiment **(a)** Settlement by offspring was not significantly affected by the food treatment. Sample sizes are; high food availability, n = 22, low food availability, n = 20. **(b)** Settlement by offspring was not significantly affected by paternity of clutches (mixed vs. single) and there was no apparent interaction effect with the food availability treatment. Sample sizes are; mixed paternity + high food, n = 5, single paternity + high food, n = 6, mixed paternity + low food, n = 3, single paternity + low food, n = 10.

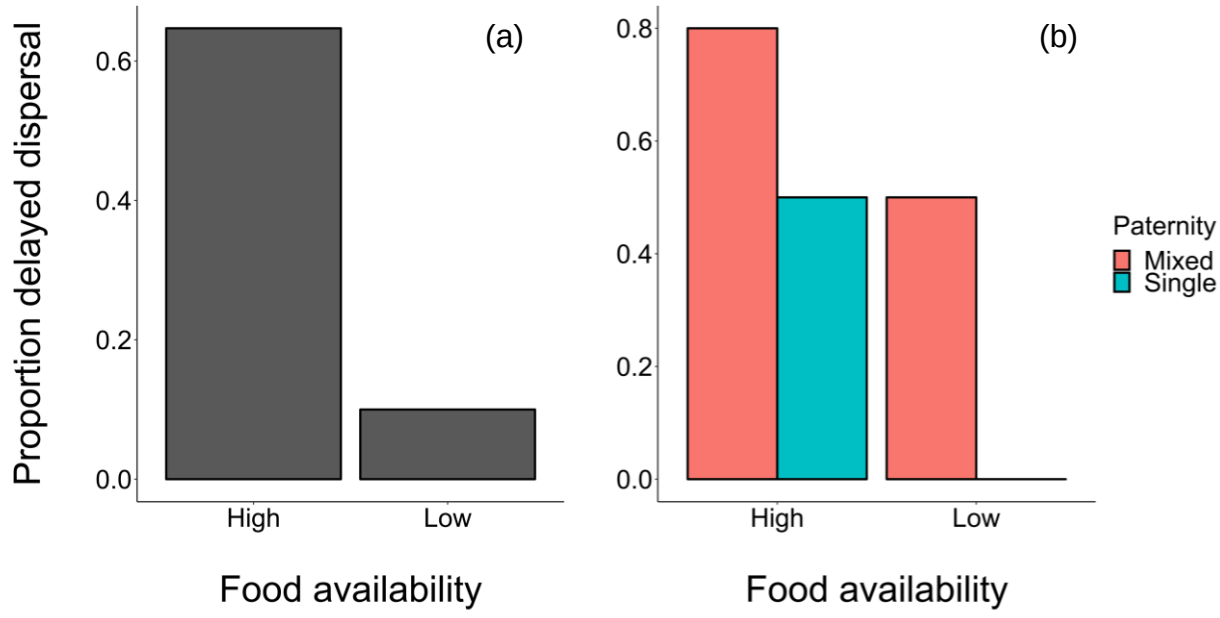

Figure 5: Delayed dispersal behaviour by *Liopholis whitii* offspring in our mesocosm experiment **(a)** Delayed dispersal by offspring was significantly affected by the food treatment. Sample sizes are; high food availability, n = 17, low food availability, n = 10. **(b)** Delayed dispersal by offspring was not significantly affected by paternity of clutches (mixed vs. single) and there was no apparent interaction effect with the food availability treatment. Sample sizes are; mixed paternity + high food, n = 5, single paternity + high food, n = 4, mixed paternity + low food, n = 2, single paternity + low food, n = 5.

Discussion

We used a multi-faceted approach to test how food availability and sibling relatedness affect sibling conflict and family structure in a facultatively family living skink. We first conducted an experiment using small-scale enclosures, each housing a single family (mother plus offspring), to test the effects of food availability and natural variation in sibling relatedness (single vs. mixed paternity clutches) on behavioural conflict between siblings. We next conducted a mesocosm experiment to test if these factors had any influence on the extent of offspring dispersal and hence family structure. Overall, there was no consistent effect of these factors across the two experiments. We did find that increased food availability increased the amount of delayed dispersal by offspring, but we were unable to specify the mechanism underlying this result. We also found no support for the idea that sibling conflict can be mediated by birthing asynchrony. We discuss our results in the context of other species that live in simple facultative family groups, and potential implications for understanding the early evolution of family living.

Under kin selection theory (Hamilton 1964), we can make two predictions about how siblings should behave towards one another under different social and environmental conditions. First, conflict between siblings is expected to be reduced when they share the same father compared to when they are from different fathers (i.e., when they are full vs. half siblings) (Hamilton 1964). Indeed, relatedness between siblings has been sown to have significant effects on sibling interactions and dispersal behaviour across a range of solitary species (e.g., Sadeh 2012; Kapranas et al. 2016; Mathiron et al. 2019), species that live in simple and facultative family groups (Dobler and Kolliker 2011; Davis 2012; Falk 2014; Ruch et al. 2014a, b) and species that live in more complex social systems (e.g., Briskie et al. 1994; Giron et al. 2004; Segoli et al. 2009a, b; Boncoraglio and Saino 2008; Boncoraglio et al. 2009; Hodge 2009; Cornwallis et al. 2010; Schultner et al. 2014; Caro et al. 2016). In contrast, while we found that sibling conflict was reduced in single vs. mixed paternity clutches in our small enclosures, these effects were minor and across both studies we failed to find any strong evidence for this effect. This could reflect that paternity of clutches has no effect on sibling conflict, or potentially an artefact of the relatively small sample sizes with which we tested paternity effects, particularly in the mesocosm experiment (e.g. for effects on delayed dispersal, single

paternity clutches; 2 delayed, 7 did not delay, mixed paternity clutches; 5 delayed, 2 did not delay).

The second prediction under kin selection theory about how siblings should behave towards one another is that when the benefit to cost ratio of helping is high, sibling conflict should be reduced (Hamilton 1964). In the case of our experimental manipulations, if resources (such as food) are abundant, there should be inclusive fitness costs, as opposed to benefits, to competing with relatives, and hence conflict should be reduced (West et al. 2002; Bourke 2014). In line with this prediction, we found that offspring dispersal was significantly influenced by food availability in our mesocosm experiment; offspring delayed dispersal to remain within their mother's home range much more when food availability was high vs. low (65% delayed dispersal when food was high vs. 10% when food was low), and there was a (non-significant) increase in offspring settling away from their home enclosure under the low food availability treatment. These effects are potentially mediated by increased levels of conflict when food availability is low. However, when we tested this explicitly in our small enclosure experiment, we found no effect of manipulating the amount of food available on sibling conflict, either immediately after birth or at three weeks of age.

There are three potential explanations underlying the contrasting results between the small enclosure and mesocosm experiments. First, this could arise if the scale of the small enclosures was such that parent-offspring resource competition was increased compared to conditions in the mesocosm experiment, overwhelming and masking any effects of food availability on sibling competition (e.g., see Schrader et al. 2015; Kramer et al. 2017; Munch et al. 2018). However, this seems unlikely here given that direct maternal-offspring aggression is virtually never observed (Botterill-James 2017b; this study) and that there was no effect of a mother's postnatal change in weight on the change in SVL or condition of her offspring. Second, sibling conflict in our system may be more complicated than appreciated, and our assays were unable to detect changes in subtle forms of sibling conflict (for example, in a study of effects of relatedness on sibling cooperation in earwigs, some components of cooperation were affected by relatedness but not others, Falk et al. 2014). Third, there may have been no effect of food availability on sibling conflict, and increased dispersal in the low food treatment may have simply occurred because when food availability is low, there is a greater

incentive for offspring to move from their mother's home site in search of better options. This is consistent with evidence from other systems, where low per capita resources increase the benefit/cost ratio and extent of offspring dispersal compared to philopatry (reviewed in Bowler and Benton 2005), including in other mesocosm experiments using lizards (e.g., Massot and Clobert 1995).

Finally, we tested whether mothers might be able to indirectly control sibling conflict via adjustment of birthing asynchrony. As our study species exhibits considerable variation in the extent of birthing asynchrony (1 to 8 days: While et al. 2007, 0 to 7 days; this study), we had expected that birthing spread may function to reduce sibling conflict via establishment of stable dominance hierarchies, analogous to the argument for the function of hatching asynchrony in birds (Hahn 1981; Roulin and Dreiss 2012). However, there was no evidence of this; although there was a trend for lower conflict with increased birth spread, this effect was minor and not statistically significant. These results are consistent to previous studies that found no effect of birth order and spread on offspring dispersal behaviour using a virtually identical mesocosm to that described here (Halliwell et al. 2017a). Combined, these results provided limited support for the argument that birthing asynchrony functions as a mechanism that allows mothers to control sibling conflict (While and Wapstra 2009).

In summary, we tested how the two key parameter of Hamilton's rule (1964) influences sibling conflict and patterns of offspring dispersal in a facultatively family living reptile. We found some evidence that sibling conflict is reduced when siblings share the same father, but no evidence that this influenced offspring dispersal. Similarly, we found no consistent effect of food availability across our two experiments; high food availability had no effect on conflict but did promote delayed dispersal of offspring. Instead, in *L. whitii*, patterns of offspring dispersal and family structure may be more dependent on father offspring interactions, with previous research showing that father-offspring conflict is high (Botterill-James et al. 2017b) and that fathers do not tolerate non-kin offspring within their home range (While et al. 2009a). Even though we found no strong evidence that sibling interactions are fundamental drivers of family living here, there may scenarios where they do have important effects (e.g., Falk et al. 2014; Ruch et al. 2014a, b). Future research that experimentally manipulates predicted influential variables and measures response at the individual and family level will allow us to

further investigate the causes and consequences of sibling interactions for family dynamics as well as other key family interactions. Ultimately, such a shift in focus from parent-offspring interactions to sibling interactions and other neglected process occurring in family life (see Kramer and Meunier 2018) will be crucial in building a more holistic and detailed understanding of the early evolution of family life.

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Supplementary information

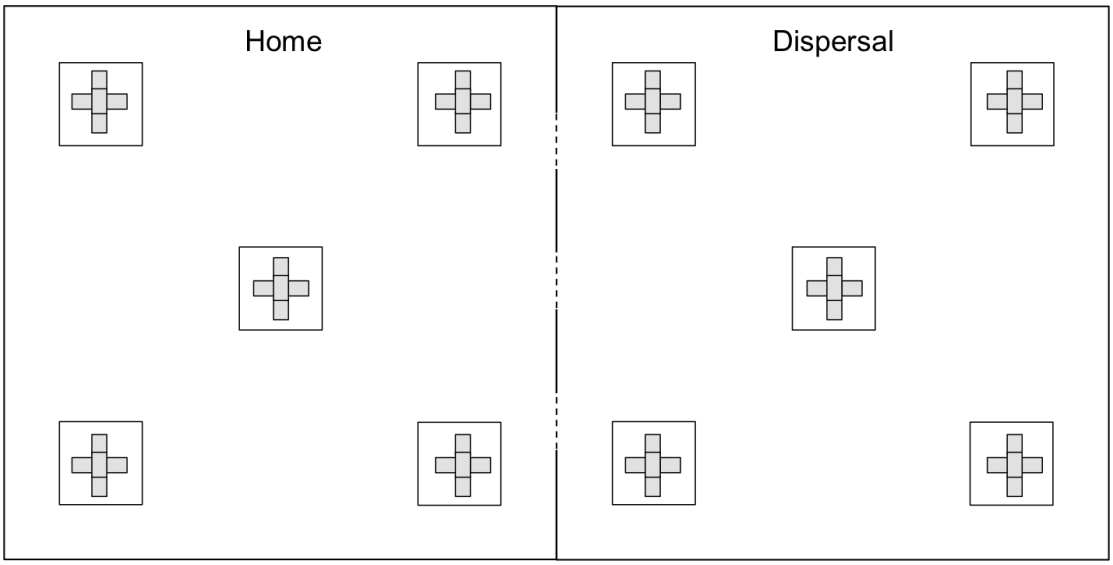

Figure S1. Diagrammatic representation of an enclosure pair showing arrangement of habitat patches. Dashed lines indicate location of the three dispersal corridors.

CHAPTER FIVE

Resource availability, but not polyandry, influences sibling conflict in the burying beetle *Nicrophorus vespilloides*

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Abstract

Conflict over resources is a fundamental component of family life. Family conflicts are predicted to be strongly influenced by resource availability and the levels of relatedness between family members. Here we examined the effects these factors have on intrabrood sibling conflict in a family living beetle where offspring are partially dependent on parental provisioning, *Nicrophorus vespilloides*. Specifically, we measured the intensity of offspring begging behavior in response to experimental manipulation of (a) relatedness between siblings (through mating females monogamously or polyandrously), and (b) resource availability (through varying amount of resources at onset of breeding). In addition, we examined the extent to which these factors influence sibling conflict directly or indirectly via maternal effects on the offspring's begging behaviour. We found no effect of polyandry on sibling conflict or patterns of female reproductive investment, but we did find that sibling conflict was influenced by resource availability. Specifically, larvae spent more time begging on smaller carcasses, but only in smaller clutches. This is possibly due to offspring being only semi-dependent on parental provisioning, with begging being ineffective relative to self-feeding in larger broods. In addition, we found that that resource availability affected patterns of female reproductive investment: when resource availability was low, females laid eggs more synchronously, and produced fewer eggs but of a larger size. We discuss potential explanations for these results, and implications of this study for understanding the factors that mediate family dynamics.

Introduction

Conflict over limited resources is a fundamental component of family life across a wide range of organisms (Mock and Parker 1997). Understanding how different biotic and abiotic factors mediate the resolution of conflict between family members and the consequences of this for the maintenance and diversification of animal societies is thus a key challenge for evolutionary biologists (Maynard-Smith and Szathmary 1995; Bourke 2011). The majority of studies have addressed this challenge from the perspective of exploring the factors that reduce intergenerational conflict. That is, the extent to which different biotic and abiotic factors mediate the costs of prolonged parental care to the parents and the benefits of care to their offspring (e.g., Emlen and Wrege 1992; Brown et al. 2010; Griffin et al. 2013). However, family life is also characterised by prolonged association between siblings (e.g., intra-generational associations) and the level of conflict and/or cooperation between siblings may be just as important as that between parents and offspring for the maintenance of family living (Falk et al., 2014; Ruch et al., 2014). Despite this, the mechanisms underpinning intrabrood social interactions and their subsequent implications for the evolution of sociality are yet to be fully investigated (Forbes 2007; Falk et al., 2014; Ruch et al., 2014).

Like other forms of family interactions, the balance of conflict and cooperation between siblings should depend on the level of relatedness between brood mates and the relative costs and benefits of conflict and cooperative behaviours (Hamilton 1964). Therefore, any factors that influence these traits should be important in mediating the extent of cooperation versus conflict between siblings and ultimately the evolutionary stability of family living. Two factors are likely to play a key role in this context. First, low levels of female polyandry (mating with multiple males) should increase relatedness between siblings, thereby promoting increased cooperation (Hughes et al. 2008; Cornwallis et al. 2010; Lukas and Clutton-Brock 2012). Second, for a given level of relatedness, resource availability should be important in mediating the costs and benefits of cooperating with group members. Specifically, as the intensity of sibling competition is largely driven by a mismatch between supply and demand for resources (Mock and Parker 1997) high resource availability should increase the costs of competing with siblings through reduced inclusive fitness, resulting in reduced levels of competitive behaviours.

Inter-generational (e.g., parent-offspring) and intra-generational (e.g., intrabrood) conflict are often thought of independently, despite the fact they are often highly dependent on one another (Mock and Parker 1997; Parker et al. 2002). Indeed, under classic parent-offspring conflict theory (Trivers 1974), conflict arises between an offspring and its parent because the offspring attempts to solicit parental resources at the expense of its siblings (current or future). In the context of intrabrood sibling conflict, the level of conflict will have consequences for a parent's fitness, leading to selection on parental mechanisms regulating conflict within a brood. Parents can achieve this via behavioral adjustment of parental care once the offspring have hatched/are born (Price and Ydenberg 1995). Alternatively, it can be achieved before offspring hatch/are born via maternal deposition of hormones or resources into the eggs or across the placenta (Groothuis et al. 2005; Meylan et al. 2012) or via maternal manipulation of offspring birthing/hatching patterns (e.g., hatching asynchrony; Hahn 1981). Therefore, to fully understand the factors responsible for variation in family conflict we need to understand the extent to which conflict and cooperation is a direct result of sibling interactions or indirect parental effects.

We empirically investigated the direct and indirect ways in which polyandry and resource availability influence levels of within-brood conflict in the burying beetle *Nicrophorus vespilloides*. Like all member of the genus *Nicrophorus*, this species breeds on vertebrate carcasses, which represent a limiting resource shared by the brood (Scott 1998). Parents provision the brood with pre-digested carrion from the carcass, resulting in sibling conflict in the form of competitive begging for access to parental provisioning (Smiseth et al. 2007a,b). Provisioning improves offspring fitness despite the fact that offspring can self-feed from the carcass (Eggert and Muller 1997; Smiseth et al. 2003). We manipulated female polyandry and resource availability in a factorial design and examined the extent to which these factors influenced the level of conflict within *N. vespilloides* broods measured as the intensity of offspring begging behaviour (see Smiseth and Moore 2002; Smiseth et al. 2003). We also measured the extent to which these effects were mediated directly through siblings recognising and competing with one another, or indirectly through maternal effects. As burying beetle mothers can potentially influence levels of post-hatching conflict between siblings via a number of routes, either through altering the level of hatching asynchrony, through pre-hatching maternal effects (in the form of increased resources invested into eggs, such as nutrients and/or hormones) or by altering the level of post-hatching care (in the form of provisioning

offspring with pre-digested carrion from the carcass). Taken together, our experiments reported here provide valuable insights into the roles female polyandry and resource availability play in mediating within-brood conflict.

Methods

To address our aims, we carried out two laboratory experiments. In the first experiment, we manipulated both levels of relatedness between siblings (by creating both monogamous and polyandrous treatments; see House et al. 2008) and resource availability (by letting females breed and raise offspring on either a small or large mouse carcass; see Smiseth and Moore 2002; Smiseth et al. 2014). We then monitored subsequent effects on female laying asynchrony, female provisioning, sibling begging behaviour and offspring growth and survival. In our second experiment, we controlled for any potential effects due to laying asynchrony, brood size and female provisioning, and compared levels of larval begging across three treatments to determine whether intrabrood conflicts were influenced by pre-hatching maternal effects or direct kin recognition. All beetles used in this study were from an outbred laboratory population maintained at the University of Edinburgh. Beetles were housed individually in clear plastic boxes (124 x 82 x 22 mm) and kept at 20 ± 2 °C under a 16:8 hr light/dark cycle. Beetles were fed small pieces of organic beef twice a week. At the start of the experiments beetles were aged 18–27 days post-eclosion.

Experiment 1

Manipulation of Relatedness and Resource Availability

We manipulated levels of relatedness between siblings by altering levels of multiple mating by allowing females to mate with either one male (monogamous treatment) or two males (polyandrous treatment). Previous mating experiments with *N. vespilloides* have successfully resulted in mixed paternity broods (see House et al. 2007; Pettinger et al. 2011; Sakaluk and Müller 2008). Pairs of unrelated (to grandparent level) virgin males and females were mated by placing them in a petri dish together. We taped the dish lids shut to avoid escape of beetles. In the polyandrous treatment, the first male was removed from the petri dish after 4 hours and replaced with a second male (unrelated at the grandparent level to both the female and her first mate) who was also left to mate with the female for 4 hours. In the monogamous treatment, pairs were left together to mate for 8 hours.

Males were briefly removed and reintroduced to their petri dish at the 4 hour mark of the mating to control for the disturbance caused when removing the first male in the polyandrous treatment. After mating had been conducted, females were transferred to a transparent container (170 x 30 x 120 mm, and 60 mm high) filled with 1 cm of moist compost and provided with a previously frozen mouse carcass (supplied from Livefoods Direct Ltd, Sheffield, UK).

We manipulated resource availability by letting females breed and raise offspring on either a small (8-12 g) or a large (21-25 g) mouse carcass. These weights were chosen based on previous work showing that *N. vespilloides* breeds on carcasses ranging in size from 1 to 40 g (Smiseth and Moore 2002) and that larvae are smaller on carcasses in the smaller range, suggesting that resources are limited in this case (Smiseth et al. 2014). The male was removed at this stage because males are less involved in resource provisioning to offspring than are females, and male assistance in provisioning of resources has no detectable effect on offspring growth or survival (Smiseth et al. 2005). In total, we had 135 beetle broods: 36 in the monogamous/high resource treatment; 32 in the monogamous/low resource treatment; 34 in the polygamous/high resource treatment; and 33 in the polygamous/low resource treatment.

Data collection

In *N. vespilloides,* egg laying starts at 21 ± 2 (mean and standard error) hours after females are given access to a carcass, and the first eggs of a given clutch start hatching on average 81 ± 3 h after females are given access to a carcass (Smiseth et al. 2006). We collected information on egg laying by placing the boxes on flat-bed scanners (Canon Canoscan 9000F Mark II, Canon Inc., Tokyo, Japan) and scanning the bottom of the breeding boxes every hour until after the completion of oviposition using Vuescan professional edition software (Hamrick Software, Sunny Isles Beach, FL, USA). Eggs are visible at the bottom of the breeding box and the visible number of eggs closely corresponds to the actual clutch size (Monteith et al. 2012). From the scanned images, we counted the number of new eggs laid each hour to determine the laying spread (the time between the first and last egg being laid) and the total number of eggs laid (clutch size). In addition, we measured egg length and width in pixels for 5 randomly chosen eggs in each clutch using the software ImageJ (Abramoff et al.2004). The measurements

were then converted into metric length (mm) and used to calculate a prolate spheroid volume, V, for each egg using the equation $V = (1/6)\pi w2L$, where w is the width and L the length of the egg (Berrigan, 1991). This data was then used to calculated an average egg size for each clutch.

Offspring begging peaks 24 hours after the first eggs start hatching (Smiseth et al. 2003). We therefore collected behavioural observations of each clutch as close as possible to 24 hours post first hatched larva (the average time from hatching of the first egg that each clutch was observed was 29 ± 0.4 h). Observations were conducted under photographic red light using instantaneous sampling every 1 min for 30 min in accordance with the protocol previously developed (Smiseth and Moore 2002; Smiseth et al. 2003; Smiseth et al. 2005). To quantify sibling competition, we counted the number of larvae in a given brood that were feeding from the parent and that were begging at each scan. An offspring was scored as feeding when there was mouth-to-mouth contact between it and the parent, and it was scored as begging when raising its head toward the parent while waving the legs when within less than the width of its pronotum from the parent or touching the parent (MacColl et al. 2003). This distance corresponds to the distance from which offspring start begging (Rauter and Moore 1999). We calculated the average percentage of time spent begging by each larva in the brood when the female was near the larvae, bi, as $bi = \Sigma b/L \times 100/d$, where Σb is the total number of begging events occurring during the 30 scans in an observation session, L is the brood size at the time of observation, and d is the number of scans during an observation session that the female was within a pronotum length distance of the offspring (Smiseth and Moore 2002; Smiseth et al. 2003).

We also recorded maternal parental behaviours during these observations (see Walling et al. 2008; Andrews et al. 2016 for similar approaches). Specifically, we estimated the amount of time females spent providing direct care, defined as when the females was provisioning food to the brood (engaging in mouth-to-mouth contact with at least one larva) or consuming carrion (manipulating carrion within the crater), versus indirect care, defined as when the female was maintaining the carcass (adding anal or oral secretions to the surface of the carcass, excavating the depression in the soil surrounding the carcass, or moving the carcass from below) or guarding the carcass (standing still in a position near the crater where she could defend the brood from

predators and conspecifics). All other maternal behaviours that occurred were recorded as non-parental behaviours (self-grooming while on the carcass, or being absent from the carcass or crypt) and were not analyzed further. We then calculated the percentage of time during the observation period that mothers spent on direct and indirect care and used these measures in our final analyses of differences in amounts of care between treatments.

Finally, we measured the consequences of our treatments for offspring fitness in terms of offspring survivorship and growth. We measured offspring survivorship by counting the number of larva present in each brood once they had dispersed from the carcass. Dispersal occurs when offspring leave the carcass and settle in the surrounding soil (typically once the carcass has been fully consumed) and is normally synchronous, approximately 144 h after the laying of the first egg in a clutch (Smiseth et al. 2007b). To measure offspring growth, we compared average larval mass between treatments (each larva was weighed individually at dispersal to the nearest 0.001 mg), with initial larval mass (weighed immediately following observations) included as a covariate.

Experiment 2

Creation of treatment groups

We conducted a second experiment to examine the extent to which differences in within-brood conflict were the result of conflict arising directly from larva recognising and competing more intensely with one another, or indirectly through pre-hatching maternal effects (for example via egg hormone levels). To achieve this, we recorded larval begging in standardised broods comprised of 10 same-aged larvae presented with a standardised stimulus in the form of a dead adult beetle (Smiseth and Parker 2008; Smiseth et al. 2010; Mäenpää et al. 2015). This protocol provides an experimental procedure for excluding confounding effects due to variation in the laying spread, and hence size and age-composition of the brood (Smiseth et al. 2003; Smiseth et al. 2007a, b) or the behaviour of adults (Smiseth et al. 2010). To generate experimental broods, we moved the breeding female and the carcass to a fresh container 65 h after first being placed on the carcass, thereby leaving the eggs to hatch in the original container. We checked the original container hourly each day for the presence of newly hatched

larvae, which we used to generate the experimental broods of 10 larvae. The experimental broods were then placed jointly on the carcass in the female's container.

We created the following treatment groups to establish the extent to which differences in within-brood conflict were the result of pre-hatching maternal effects (other than laying asynchrony) or a direct result of differences in relatedness. In the first treatment group, all 10 larvae were from a single mother from a monogamous mating (single origin clutch) (n = 17 broods). In the second treatment group, we combined 5 larvae from each of two mothers unrelated at the grandparent level (hereafter 'mixed origin' clutch) (n = 17). In the third treatment group, all 10 larvae were from a single mother from a polygynous mating (single origin clutch) (n = 15). Resource availability was equal across these treatments at 8–12 g, as we expected that this condition would produce the highest levels of conflict and allow us to detect any treatment effects.

Data Collection

We recorded larval begging 24 h (± 20 min) after the experimental broods had been generated, to coincide with the stage in larval development when begging peaks (Smiseth et al. 2003). Thirty minutes before we started recording larval begging, we removed the adult beetle to be used as a stimulus. This was always a breeding female who was unfamiliar to any larva in the clutch. We then killed the beetle by placing her in a -20°C freezer for 20 min and left her to thaw for another 5 min before pinning her within a small plastic container (12 x 8 cm x 2 cm) lined with a moist paper towel (Smiseth et al. 2010). The female was pinned in a position mimicking that of a parent regurgitating food. Once the female had been pinned, we removed the larvae and placed them next to the pinned female. We waited 5 min before starting the observations to give the larvae time to settle. We recorded larval begging using instantaneous recording every 1 min during a 30-min period, and for use in the analyses, we calculated the average percentage of time spent begging by each larva in the brood when within a pronotum width distance of the dead adult female (as described for experiment 1).

Statistical analyses

Data were analysed using ANOVA (type III) and General Linear Models implemented in R version 3.3.0 (R development core team 2016) through the 'aov' and 'glm' functions.

In the first experiment we ran models examining differences in variables relating to (1) female patterns of reproductive investment (time to laying, clutch size, average egg size, laying spread), (2) social interactions within the family (average percentage of time spent begging by each larva in the brood, percentage of time spent on direct and indirect care by mothers), and (3) offspring fitness (number of larvae at dispersal, average individual larval mass at dispersal). Mating treatment (monogamous vs. polygynous), resource treatment (low vs. high) and their interaction were entered as fixed factors in each of these models. Clutch size was included as a covariate for models of laying spread and offspring survival, average larval mass at the time of observation was included as a covariate for the model for larval mass at dispersal, and number of larvae at observation was included as a covariate for modelling average percentage of time spent begging. Time elapsed from hatching until the observation was not equal for all broods, but inclusion of this variable as covariate when analysing time begging did not affect any model outputs, so was removed from the final model. For the second experiment, we ran a one-way ANOVA to analyse differences in average percentage of time spent begging by each larva between our three treatment groups.

All models started with a full set of interactions between dependent variables, and we subsequently eliminated non-significant ($p > 0.05$) interaction terms. We report results for models containing all main effects and significant interactions following backward elimination of non-significant interactions. All data were checked for violation of assumptions, and where required, transformed to fit assumptions. Additionally, two models violated assumptions of normality (models examining levels of larval begging in Experiments 1 and 2) due to the presence of an outlier more than two standard deviations away from the mean; after removal of the outlier, each model conformed to assumptions and removal of the outlier did not affect the model's overall interpretation, so results for these models are reported with outliers removed. The model analysing number of larvae at dispersal from Experiment 1 was highly overdispersed but corrected by running a negative binomial model.

Results

Experiment 1

Effects of polyandry and resource availability on female reproductive investment

Of the 135 females mated, 104 successfully laid eggs, with equal success across treatments (Mating treatment: $Z_{1, 120} = -0.75$, $P = 0.45$, Resource treatment: $Z_{1, 120} = 0.81$, $P = 0.42$, Mating x Resource: $Z_{1, 119} = -0.83$, P = 0.41). The average clutch size for the experiment was 26.36 ± 1.43, consistent with findings in other studies (e.g., Müller et al. 1990).

We found no significant differences between monogamous and polyandrous females in the time elapsed between females being placed on the carcass and the laying of their first egg, the number of eggs laid, egg size or in the laying spread (Table 1). We also found no effect of resource availability on the time elapsed between females being placed on the carcass and the laying of their first egg (Table 1). We did, however, find a significant effect of the resource treatment on number of eggs laid and on egg size (Table 1). Specifically, females appeared to alter the trade-off between the number and size of eggs depending on resource availability, with a greater number of eggs of smaller size laid in the high resource treatment compared to the low resource treatment (Figure 1). Resource availability also had a significant effect on laying spread, with females laying over a longer time period in the high resource treatment (25.96 ± 1.42 h, n = 54) compared to the low resource treatment (18.92 ± 1.41 h, n = 50) (Table 1).

Table 1. Outputs of models examining treatment effects on female reproductive decisions. Significant effects are bolded.

Trait	Time taken to lay first egg (hrs)	Clutch size	Average egg size (mm^3)	Laying spread (hrs)
Mating treatment	$F_{1,109} = 1.98$, P = 0.16	$F_{1,101} = 1.46$, P = 0.23	$F_{1,96} = 0.37$, P = 0.54	$F_{1,100} = 0.87$, P = 0.87
Resource treatment	$F_{1,109} = 0.72$, P = 0.40	**$F_{1,101} = 9.25$, P<0.01**	**$F_{1,96} = 4.37$,P = 0.04**	**$F_{1,100} = 5.29$, P = 0.02**
Mating x Resource	$F_{1,108} = 0.03$, P = 0.86	$F_{1,100} = 0.02$, P = 0.88	$F_{1,95} = 0.36$,P = 0.55	$F_{1,97} = 0.01$, P = 0.93
Clutch size				**$F_{1,100} = 25.31$, P<0.01**
Resource x clutch size				$F_{1,97} = 0.63$, P = 0.43
Mating x clutch size				$F_{1,97} = 1.30$, P = 0.26

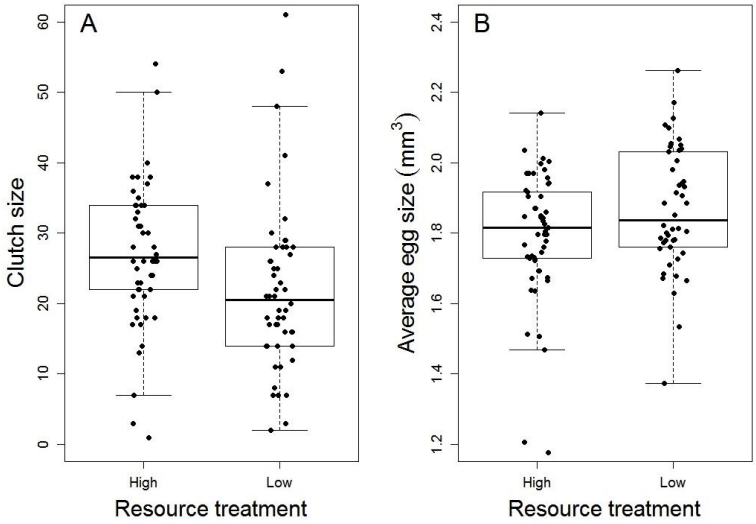

Figure 1. Differences between high and low resource availability in female clutch size (A) and average egg size (B). Centre lines represent medians and error bars represent 95 % confidence intervals. For (A); n = 54 (high resource treatment) and 50 (low resource treatment), for (B); n = 53 (high resource treatment) and 46 (low resource treatment).

Effects of polyandry and resource availability on social interactions within the family

Across all treatments, larvae spent 16.78 ± 1.49 % of their time, on average, begging (n = 78). Overall, we found no significant effect of mating treatment on time spent begging by larvae (Table 2). However, there was a significant interaction effect between resource treatment and brood size on time spent begging by larvae (Table 2). Specifically, there was no effect of brood size on the amount of time larvae spent begging in the high resource treatment, but begging decreased with an increase in brood size in the low resource treatment (Figure 2). We found no effect of mating or resource treatment on the amount of time a female spent on direct and indirect maternal care (Table 2).

Table 2. Outputs of models examining treatment effects on offspring begging behaviour and direct and indirect forms of female parental behaviour. Significant effects are bolded.

Trait	Time begging (%)	Time spent on direct care (%)	Time spent on indirect care (%)
Mating treatment	$F_{1,72} = 0.09$, P = 0.77	$F_{1,92} = 1.42$, P = 0.24	$F_{1,91} = 1.14$, P = 0.29
Resource treatment	**$F_{1,72} = 6.46$, P = 0.01**	$F_{1,92} = 1.04$, P = 0.31	$F_{1,91} = 0.45$, P = 0.50
Mating x Resource	$F_{1,70} = 0.03$, P = 0.86	$F_{1,90} = 0.27$, P = 0.60	$F_{1,90} = 0.47$, P = 0.49
Clutch size	$F_{1,72} = 0.01$, P = 0.91		
Resource x clutch	**$F_{1,72} = 5.90$, P = 0.02**		
Mating x clutch size	$F_{1,70} = 0.13$, P = 0.72		

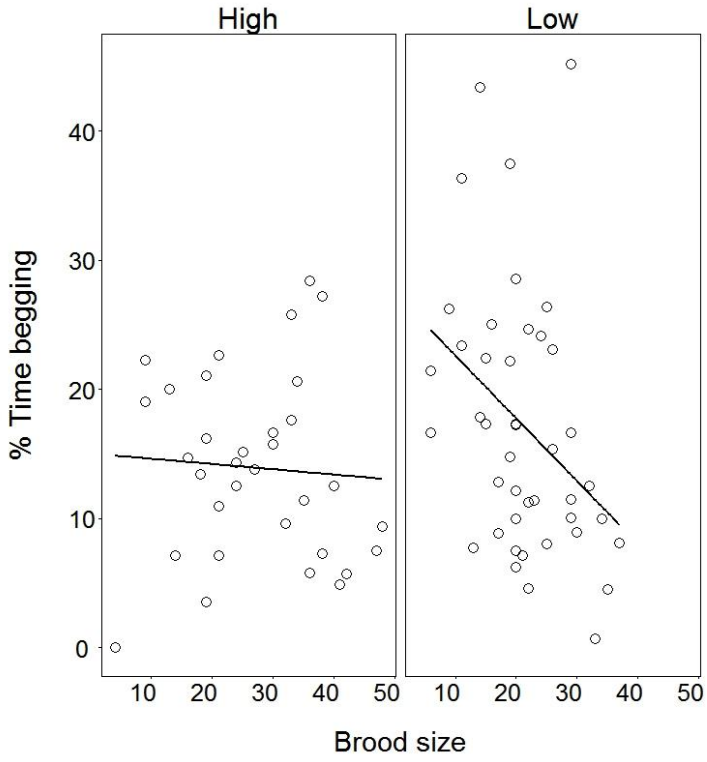

Figure 2. Effect of brood size on offspring begging behaviour in high and low resource treatments. n = 33 (high resource treatment) and 43 (low resource treatment).

Consequences of polyandry and resource availability for offspring fitness

Across all treatments, the average number of offspring surviving to dispersal was 18.08 ± 0.99, with an individual larval mass of 0.173 ± 0.004 g. We found no effect of the mating treatment on larvae number and size at dispersal (Table 3). Resource treatment also had no effect on larval number at dispersal (Table 3). However, offspring in the high resource treatment had a significantly greater mass at dispersal than offspring in the low resource treatment (Table 3, Figure 3).

Table 3. Outputs of models examining consequences of treatments for offspring survival and growth. Significant results are bolded.

Trait	Number of larvae at dispersal	Average mass at dispersal
Mating treatment	$Z_{1,77}$ = 0.17, P = 0.87	$F_{1,80}$ = 0.09, P = 0.77
Resource treatment	$Z_{1,77}$ = -0.14, P = 0.89	**$F_{1,80}$ = 5.82, P = 0.02**
Mating x Resource	$Z_{1,74}$ = 0.30, P = 0.76	$F_{1,77}$ = 0.20, P = 0.66
Clutch size	$Z_{1,77}$ = 1.43, P = 0.15	
Average mass at observation		$F_{1,80}$ = 1.18, P = 0.28

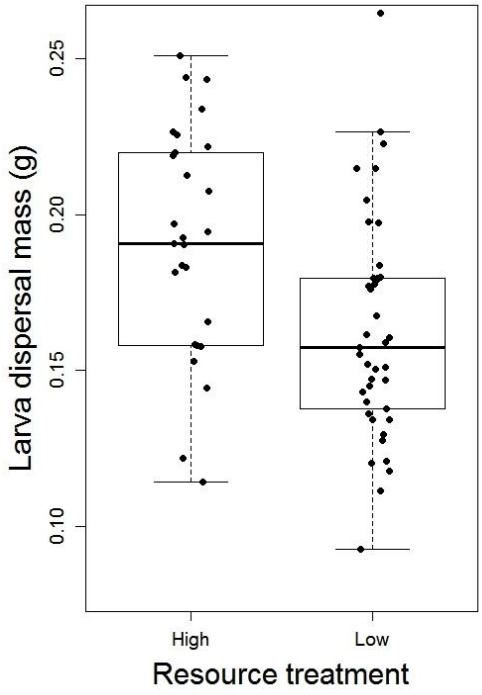

Figure 3. Effect of resource treatment on larval mass at dispersal. Centre lines represent group medians and error bars represent 95 % confidence intervals. n = 40 (high resource treatment) and 48 (low resource treatment).

Experiment 2

Across treatments groups, the average percentage of time spent begging by each larva in the brood was 21.63 ± 2.18 % (n = 47). Overall, we found no significant differences in the proportion of time larvae spent begging between the three treatment groups ($F_{2,43}$ = 1.48, P = 0.24).

Discussion

Factors that influence the costs and benefits of interacting with family members, such as relatedness between group members and resource availability, are predicted to influence the balance of cooperation and conflict between siblings and ultimately the evolutionary stability of family living. We tested this hypothesis by manipulating polyandry and levels of resource availability in a family living beetle with prolonged parent-offspring and sibling-sibling association. We found no support for an increase in conflict between brood mates as a result of female polyandry. Furthermore, we found only limited support for an increase in conflict when resource availability was limited. Combined these results provide no support for the

role of relatedness in mediating the outcome of family conflicts, while there was some support for a role of resource availability.

Effects of polyandry and resource availability on female reproductive investment

We found no effect of relatedness between family members (manipulated through female polyandry) on any of our four variables relating to patterns of female reproductive investment (onset of egg production, clutch and egg size, laying spread). This is in contrast to work on a range of species that has shown a number of direct benefits of polyandry. For example, a meta-analysis of 78 insect species found that polyandry increases lifetime egg production via higher fertilisation success (Arnqvist and Nilsson 2000, see also Simmons 2005; Taylor et al. 2014). Polyandry has also been shown to speed up reproductive events as evidenced by the positive effect of polyandry on clutch production in the ladybird beetle *Eriopis connexa* (Colares et al. 2015) and faster incubation times across birds (Lloyd and Martin, 2003). One potential explanation for a lack of effects here is that the direct benefits of polyandry are likely to elicit relatively small effects sizes (Slatyer et al. 2012) that may not have been picked up by our experimental design. Thus, additional work is required to explore the potentially more subtle mechanisms by which female polyandry may directly increase female reproductive success and/or influence female patterns of reproduction.

We found strong effects of resource availability on female reproduction. Specifically, while resource availability had no effect on the timing of onset of egg production, it did influence how females balanced the trade-off between the number and size of eggs, with females on larger carcasses producing more eggs but of a smaller size than females on the smaller carcasses. These results are in line with previous literature on *N. vespilloides* which showed a reduction in clutch size on carcasses of 10 g or lower (Müller et al. 1990). While we were unable to examine whether this resulted in equivalent investment overall (because we did not measure total brood mass at the egg stage), these results suggest that resource availability influences prenatal reproductive decisions. Previous work examining the trade-off between the number and size of offspring in *N. vespilloides* found that females breeding on larger carcasses produced both more and larger offspring (Smiseth et al. 2014), whereas we found females breeding on larger carcasses produced more offspring of a smaller size. A potential

reason for these contrasting results is that previous studies measured offspring number and size when larvae dispersed from the carcass (corresponding to the end of the parental care period), whereas our measurements were taken at the egg stage. Regardless, when we measured offspring mass and number at dispersal our results still differed from Smiseth et al (2014); we found heavier, but not more larvae on large carcasses. These differences from previous work may therefore reflect facultative adjustment of females to some other aspect of our experimental design.

We also found that females increased their laying spread when on larger carcasses. As laying spread corresponds with hatching spread in burying beetles (Smiseth et al. 2006), this potentially suggests an adaptive adjustment in hatching spread in response to resource availability, as has been suggested for hatching asynchrony in birds (e.g., Wiebe 1995; Mock and Parker 1997). Specifically, increased hatching asynchrony in burying beetles increases asymmetric competitive abilities among the brood, with later hatched offspring begging more but growing less than earlier hatched offspring (Smiseth et al. 2008). This may facilitate adaptive brood reduction under stressful environmental conditions (i.e., Lack's (1947) adaptive brood reduction hypothesis). However, our results were in the opposite direction to those predicted under the brood reduction hypothesis, in that laying spread was greatest on larger carcasses where resource availability was high (rather than on smaller carcasses as predicted by the adaptive brood reduction hypothesis). This is perhaps unsurprising, because it is unlikely that Lack's brood reduction hypothesis would apply to *N. vespilloides* for two reasons. First, females provide offspring with resources from a carcass obtained prior to egg laying, and therefore the amount of resources available to offspring after hatching is predictable at the time of laying. Thus, there should be no need for brood reduction as females could manipulate brood size by adjusting egg number rather than through hatching asynchrony. Second, parents can reduce brood size through filial cannibalism (Bartlett 1987; Müller et al. 1990), which would be more energetically efficient than the alternative of reducing brood size through establishing asymmetric sibling hierarchies. Further work is clearly needed to determine why we found an increased laying spread on a larger carcass.

Effects of polyandry and resource availability on social interactions within the family

We found no effects of female polyandry on intrabrood conflict (measured by levels of larval begging) in either of our experiments. This suggests that polyandry does not influence intrabrood conflict in *N. vespilloides*; neither through pre-hatching nor post-hatching maternal mechanisms (maternal investment in eggs or post hatching care) nor through directly mediating offspring behaviour. This is despite the strong theoretical prediction that polyandry should affect the extent of intragroup conflict via its effects on intragroup relatedness (Hamilton 1964; Hughes et al. 2008; Cornwallis et al. 2010; Lukas and Clutton-Brock 2012). Indeed, a large comparative study across birds found a positive correlation between the level of female polyandry in bird clutches and the extent of offspring begging behaviour (Briskie et al. 1994). There are several potential explanations for this lack of effect observed here. First, our manipulation of polyandry may not have resulted in mixed paternity broods, due to post-copulatory processes that bias paternity towards one male, such as cryptic female choice or sperm competition. Thus, brood relatedness may have been high across both monogamous and polyandrous treatments. However, this cannot totally explain our results given that we also found no effect when we created artificially mixed paternity broods via cross fostering in the second component of the experiment. Second, our manipulations may have worked in creating broods with different levels of relatedness but offspring may lack the relevant mechanisms to assess kin and respond accordingly. Indeed, while offspring recognition of adults has been shown to occur in burying beetles (Mäenpää et al. 2015), there is no prior information on offspring-offspring kin recognition in this system. Our results from experiment two, where our experimentally mixed broods had the same amount of larval begging as single origin broods, indicates that kin recognition of fellow larvae does not occur. This could reflect limited selection for kin discrimination in this system. In wild broods of *N. vespilloides*, brood mixing may be low, with one study finding that on average, 93 % of offspring within a brood are sired by the resident male on the carcass (Muller and Eggert 1989). This should result in relatively low variance both within and between broods in relatedness, and subsequently overall weak selection on kin discrimination and adjustment of behaviour with respect to kinship (Cornwallis et al. 2009; Cornwallis et al. 2010). This could be empirically tested in the future in *N. vespilloides* using experimental lines evolved under low, moderate and high levels of polyandry (see House et al. 2009; Schrader et al. 2015 for examples of experimental

evolution approaches in *N. vespilloides*), and testing the prediction that kin discrimination operates under moderate but not low or high levels of polyandry.

We found evidence that resource availability influenced levels of intrabrood conflict as more larval begging occurred on a smaller mouse carcass. However, this was only observed for smaller brood sizes. This appears counter intuitive, as levels of competition should be relatively lower in small broods compared to large broods. A potential explanation for these results arises from the fact that *N. vespilloides* offspring are only partially dependent on parents for food and that they can self-feed (Smiseth et al. 2003; Capodeanu-Nägler et al. 2016). Thus, at higher brood sizes, there is likely a limit to the amount of provisioning the mother can provide to offspring which reduces the effectiveness of begging, leading to more self-feeding behaviour by offspring. This argument mirrors Trumbo's (1992) explanation for between-species patterns of larvae dependence on parental feeding: in species that rear large broods, larvae are selected to maintain their independence for feeding, because parents cannot attend to each larva as well as parents in species where brood sizes are smaller. Further work is required to confirm if this mechanism is responsible for our results here. This could potentially be tested in future studies by observing levels of offspring begging in response to manipulating brood size on a fixed carcass size (i.e., altering offspring density).

Consequences of polyandry and resource availability for offspring fitness

Given our finding that polyandry had no effect on intrabrood competition (which could decrease offspring fitness), it is unsurprising that we did not observe a decrease in offspring fitness in response to polyandry. Similarly, although females could increase the fitness of their offspring by biasing paternity towards males of genetic compatibility or quality, this does not occur in *N. vespilloides* (House et al. 2011). Thus, it is unsurprising that we did not observe an increase in offspring fitness. In contrast, resource availability was found to influence offspring growth but not survival, with offspring raised on larger carcasses growing better than those on small carcasses. Our finding of increased growth on larger carcasses is intuitive and matches previous findings in this and other species of *Nicrophorus* (Bartlett and Ashworth 1988; Scott and Traniello 1990; Trumbo 1992; Eggert and Muller 1997). However, previous work on *N. vespilloides* found lower survival on smaller carcasses (Smiseth et al. 2008), which

contrasts our result of no effect of carcass size on larval survival. The contrasting results between studies may arise from the smaller carcass size used in the previous study (5 g in Smiseth et al. (2008) versus 8-12 g here) and a non-linear relationship between carcass size and larval survival; i.e., there may be a threshold carcass size between 5-8 g at which decreasing carcass size negatively impacts larval survival, however further empirical tests are required to confirm this.

Broader implications

Here, we tested for facultative adjustments of mothers and their offspring to changes in resource availability and polyandry, to gain insights into the role of these factors on family living. We found that, under restricted resource availability, there was reduced begging in larger broods, indicating the potential for sustained changes in resource availability to lead to evolutionary change in family dynamics. By contrast, we found no responses of family members to polyandry. Despite finding no facultative response of mothers or their offspring to polyandry, it is important to note that polyandry still could lead to responses over evolutionary timescales. To detect such responses in future studies will necessitate the use of comparative and/or experimental evolution approaches.

While we found no evidence that females adjust patterns of reproductive investment or parental behavior in response to polyandry, it is important to consider female responses when examining how males respond to polyandry in species where biparental care occurs. The majority of studies investigating the role of female polyandry in mediating family dynamics have focused on facultative adjustment in male care (e.g., Griffin et al., 2013), with relatively little consideration of the potential for adjustments in maternal care either pre- or post-hatching/birth. However, such female adjustments could have substantial consequences for our broader understanding of family dynamics. For example, observations of facultative adjustment of male care in response to polyandry may simply be a correlated response to changes in pre- or post-hatching female care, rather than a change driven by selection on male behaviour *per se*. Similarly, females could alter the offspring's competitive environment in response to polyandry (e.g., chemical cues of maternal condition affect intrabrood cannibalism in earwigs (Wong et al., 2014)), which could subsequently influence male care. Therefore,

future studies need to broaden their scope beyond focusing on males to accurately unequivocal identify the processes through which polyandry may influence family dynamics.

In our second experiment, we found no difference in the begging behaviour of mixed versus single origin broods. This result has implications for the use of cross-fostering experiments. Such experiments typically involve mixing offspring from broods of separate mothers (e.g., 'reciprocal cross-fostering'; see Riska et al. 1985). This key experimental design is used in range of systems to address a wide array of questions (e.g., MHC disassortative mating in mice (Penn and Potts 1998); environmental and genetic effects on immune and stress responses in birds (Brinkhof et al. 1999; Losdat et al. 2014)). However, if offspring can recognise kin and non-kin they could adjust their behavior or physiology accordingly, affecting the biological plausibility and hence interpretations of such cross-fostering experiments. Our results here suggest that this is not an issue for *N. vespilloides*, but we highlight the need for this issue to be considered in other study systems employing cross-fostering designs.

Conclusions

We conclude by echoing recent suggestions (Parker et al. 2002; Royle et al. 2012; Falk et al. 2014) for a shift in social evolution studies from isolating and considering only dyadic interactions (in particular those between parents and offspring) to testing how environmental and social factors (i.e., both the relatedness and cost/benefits terms in Hamilton's rule) influence all family members simultaneously. Such approaches moving forward will help us build a more holistic understanding of the evolution of families and social living.

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CHAPTER SIX

Hatching asynchrony in birds: unravelling the consequences for offspring and parents

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ABSTRACT

Hatching asynchrony, the process by which clutches of eggs hatch over a span of multiple days, occurs in many bird species but often results in the death of the last hatched offspring. This puzzling phenomenon has received much theoretical and empirical attention over the last few decades, but there is still little consensus as to the adaptive nature of asynchronous hatching patterns. Here, we aimed to address this question by using a yet unutilised approach to this question – a series of meta-analyses and a comparative phylogenetic analysis. Specifically, we compared the fitness outcomes of asynchronous and synchronous clutches for both parents and offspring, and the dependence of these fitness outcomes on resource availability and predictability. In our comparative analysis we tested whether these same variables predicted variation in hatching span across 50 species of birds. We found that hatching asynchrony increases parental fitness by reducing their parental effort, but at a cost to last hatched offspring. These effects were not influenced by resource conditions. Similarly, despite great variation in hatching span occurring across the species included in our comparative dataset, resource conditions did not account for this variation. These results suggest that independently of environmental conditions, hatching asynchrony helps parents keep down costs of parental care, but at the detriment of last hatched offspring, and as such, can be generally understood as a mechanism mediating within family conflicts.

INTRODUCTION

Hatching asynchrony is the process by which offspring of a given reproductive event hatch over an extended period of time, usually from hours to days (Magrath 1990; Mainwaring et al. 2014). This behaviour is common in birds and usually leads to size hierarchies forming in the clutch, whereby the first hatched offspring is larger and more dominant than their later born siblings (Dunlop 1913; Lack 1947; Bryant 1978; Morandini and Ferrer 2015). These dominance and size hierarchies can lead to the death of subdominant offspring because of starvation imposed by the older siblings (reviewed in Mock and Forbes 1994; Mock and Forbes 1995). Hatching asynchrony is therefore an interesting and puzzling biological phenomenon; it appears to have significant fitness consequences (see below), and often causes high mortality of last hatched offspring despite being under some degree of parental control via incubation behaviour.

To address this biological puzzle, several adaptive explanations have been proposed, tested empirically, and reviewed (e.g., Lack 1947; Hahn 1981; Clark and Wilson 1981; Magrath 1990; Mock and Forbes 1994; Horak 1995; Slagsvold et al. 1995; Stoleson and Beissinger 1995; Stenning 1996). Such explanations have primarily focussed on potential fitness gains from the perspectives of parents. Classically, it has been suggested that hatching asynchrony allows for efficient brood adjustment when parents are unable to predict post-hatching conditions (Lack's brood reduction hypothesis/ Resource tracking hypothesis: Lack 1947; Mock and Parker 1986; Magrath 1989; Forbes 1991; Pijanowski 1992; Mock and Parker 1995). Under this scenario, while hatching asynchrony is beneficial for parents, from the perspective of marginal offspring, hatching asynchrony is detrimental, and therefore might be viewed as a mechanism mediating parent-offspring and sibling conflict (Horak 1995; Roulin and Dreiss 2012). Other explanations have suggested that hatching asynchrony might provide parents with more direct fitness gains, by spreading out the total food demand of the brood (Ingram 1959; Hussell 1972) and hence reduce parental effort without necessarily promoting offspring mortality. This is just the tip of the ice berg, with a total of 17 hypotheses in all that have been generated in the literature (reviewed in Stoleson and Beissenger 1995). Despite the substantial amount of work carried out to test these alternative hypothesis, none have received enough quantitative or qualitative backing to

gain unified support. There is therefore no scientific consensus as to the adaptive nature of hatching asynchrony.

One reason for this lack of consensus and the large proliferation of different explanations for hatching asynchrony (Stenning 1996) is that hatching asynchrony can potentially influence parental and/or offspring fitness in a variety of context dependent ways. To explore the adaptive function of hatching asynchrony, we perhaps need to approach the question form a more generalised and simplified perspective. That is, instead of testing a single or limited set of hypotheses in a taxon specific manner, a more powerful and conceptually simplified approach is to ask; across species, what are the fitness consequences of hatching asynchrony for parents and their offspring, and does this change depending on variation in basic environmental predictors? This may allow us to not only test the efficacy of previous hypotheses, but also potentially provide a basis from which new and more encompassing, hypothesis can be generated.

Here, we collated studies that manipulated hatching asynchrony, and extracted data from these studies to examine the fitness consequences of hatching asynchrony for parents and offspring using meta-analyses. Specifically, we examined three key ways in which hatching asynchrony may influence parental or offspring fitness. First, we tested whether hatching asynchrony influences offspring fitness by examining whether hatching order (first vs. last) predicts offspring fitness (survival or growth) between asynchronous and experimentally synchronised broods. Second, we tested whether hatching asynchrony increases parental fitness in terms of increasing the number offspring surviving to fledging or mean level clutch growth, by comparing these outcomes between asynchronous and synchronised broods. Third, we tested whether hatching asynchrony increases parental fitness in terms of reduced parental effort, by comparing parental effort between asynchronous vs. synchronised broods. We also collated data on precipitation amount and predictability, as proxies for resource availability and predictability respectively, to test whether any effects of hatching asynchrony on parental or offspring fitness are dependent on these environmental parameters. Finally, we combined the above with a comparative analysis, to test whether the degree of hatching asynchrony across species and populations is predicted by variation in resource supplies (again, using precipitation predictability and amount as proxies of resource predictability and availability).

METHODS

Meta-analyses testing fitness consequences of hatching asynchrony for offspring and parents

Literature search and data collection

We report our systematic literature search following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Moher et al. 2009, see Fig S1 for PRISMA flow diagram of study search and inclusion process). We searched for papers that experimentally manipulated hatching asynchrony to allow us to estimate the difference in fitness outcomes for asynchronous vs. synchronised broods for both offspring and parents. Specifically, on April the 20th 2018, we carried out a 'Topic' search from 1945 – April 20 2018 of the *ISI Web of Science* using the following keywords: ('hatch* asynchrony' OR 'asynchronous hatching' OR 'hatch* spread' OR 'hatch* span' OR 'hatch* interval') AND ('fitness' OR 'mortality' OR 'survival' OR 'recruitment' OR 'growth' OR 'fledg*' OR 'beg*'). This yielded a total of 2371 results. We then carried out a second search in *Scopus* using the same keyword string across all years searching again the 'Article title, Abstract, Keywords'. Our Scopus search yielded another 248 results. After removing duplicates, we screened 2554 title and abstracts of individual articles, and we identified 97 potentially suitable studies where we read full-texts to assess the extent to which they could be included in our analysis (Table S2).

For a study to be included in our analysis, it had to provide a pairwise difference in either parental or offspring fitness between an asynchronous vs. experimentally synchronised clutch. For offspring fitness, we collected data – on growth and/or survival – at the individual offspring level between first and last hatched offspring. For parental fitness, we included number of offspring fledged, growth of offspring (in mass or size – e.g., wing length) and traits related to parental effort including frequency of visits to the nest, change in parent condition, and basal metabolic rate (see Table S1 for full list of specific traits recorded as part of the meta analyses).

The experimental manipulations were all applied such that synchronous clutches were created (typically by moving either eggs or nestlings between nests) and were compared to control clutches, i.e., clutches that naturally hatched asynchronously. Some studies

controlled for confounding factors due to experimental manipulation (e.g., controlled for egg handling in the synchronised clutches by also handling control eggs) which we also recorded. In addition, approximately 10 percent of our effect sizes were from comparisons of experimentally synchronised clutches with clutches manipulated to have an exaggerated degree of asynchrony (i.e., a hatching span greater that what is known to occur naturally for that species). We took only comparisons between control (naturally asynchronous) to synchronised clutch treatments and excluded any comparisons with exaggerated asynchrony-synchronised clutches where possible. If only exaggerated asynchrony-synchronised clutch comparisons were available from a a study, we took the comparison where the asynchronous treatment had a hatching span closest to what occurs naturally for that population (data on natural hatching span for the population/species was collected either from the paper itself, or from Del Hoyo et al.'s Handbook of the Birds of the World (2018)). To ensure that the inclusion of exaggerated asynchrony-synchronised comparisons did not influence our results, we ran our a model on a subset of data (data on fitness consequences of hatching asynchrony for number of offspring fledged) with these comparisons excluded; exclusion of these data did not change our inferences, and so we report our results for all models with these data included (see section 5.1 of supplementary code).

After applying these inclusion criteria, we retained 36 primary studies for our analysis; from these studies, we excluded marine species (n = 5 studies, see Table S2), as hatching asynchrony is likely under different selection pressures for these species and proxies of resource conditions are different from those for terrestrial species (see below for more details on collection of climatic data). From the studies collated, we extracted proportions, means, standard deviations and sample sizes for each relevant asynchrony-synchrony treatment comparison, and constructed separate datasets to test each of the three key ways in which hatching asynchrony may influence both parental and offspring fitness, as outlined in the introduction; (1) whether hatching asynchrony influences offspring fitness – by testing whether hatching order (first vs. last) predicts offspring fitness (survival or growth) between asynchronous and experimentally synchronised broods, (2) whether hatching asynchrony increases parental fitness in terms of increasing the number offspring surviving to fledging or brood growth, by comparing these outcomes between asynchronous and synchronised broods, and (3) whether

hatching asynchrony increases parental fitness in terms of reducing parental effort, by comparing parental effort between asynchronous vs. synchronised broods.

Often, multiple effect sizes were taken from a single study (average number of effect sizes per study in each prediction's dataset = 1.75); however, we ensured independence of data by extracting statistics to be used for multiple effect sizes from samples of independent individuals. For example, several studies included multiple estimates on offspring size and growth across the experimental period, providing data on wing length, tarsus length and/or offspring mass. Where this occurred, we preferentially included data on wing or tarsus length over mass; this is because variation in body water content can decrease the reliability of body weight as an indicator of size, a problem that does not apply to measurements of wing or tarsus length (Ricklefs 1968). For all growth measures, we only included actual estimates from raw data and did not include any estimates extracted from models (e.g. data on growth taken from growth model estimates). Where studies measured the same individual multiple times across the experimental period we took the measurements closest to fledging as our estimate of growth, because these measurements should be more biologically meaningful indicators of an offspring's reproductive potential. For our data testing consequences of hatching asynchrony for offspring, we prioritised traits related to survival over traits related to growth or size, to avoid non-independence from taking multiple effect sizes from the same individuals (we did not construct separate datasets testing offspring fitness in terms of survival and growth due to sample size constraints).

Using the above criteria, each prediction's dataset was limited in its level of non-independence, with the only source of non-independence coming from studies reporting on the same set of individuals but in different years (19% of all data across all predictions). To ensure this not influence our results, we ran a model using the dataset on fitness consequences of hatching asynchrony for number of offspring fledged both with and without these data removed; inferences were the same when the non-independent data were included, so we report our results from datasets with these data included (see section 5.2 in supplementary code).

Effect size calculation

Most of our data (77%) were reported as means with errors (e.g., number of offspring fledged, offspring growth, number parental visits to the nest to feed offspring), and so, we calculated standardised mean difference (Hedge's *d*) for the comparisons of asynchronous clutches to synchronous clutches (Borenstein et al. 2009). For proportion data (e.g., % survival) we did the same using logs odds ratios (*lnOR*). We then converted log odds ratios to its Hedges d equivalent so that effects across studies could be compared (Borenstein et al. 2009). Each effect size was a pairwise difference between an asynchronous clutch and a synchronous clutch; standardising effect sizes in this way allows for one to compare trait types that vary in their unit of measurement across studies as they are all placed on a similar scale and are of the same type. In all cases, the mean trait value or proportion for the synchronous clutch was subtracted from the asynchronous. For our data on offspring and parental fitness, measured in terms of individual offspring or mean clutch level survival or growth, a positive effect size represents higher fitness in asynchronous vs. synchronised clutches. For our model testing consequences of hatching asynchrony for parental effort, a positive value represents higher effort for parents with asynchronous clutches.

Collection of climatic data and moderator variables

We collected data on two key climatic traits; the amount of precipitation during the breeding season of the year/s of the study for each given population and the long-term predictability of precipitation for each population (a composite measure of within and between year predictability – see below for more detail). We used these metrics as estimates of resource availability and predictability for each effect size. Hence, this allowed us to test in our meta-analytical models (see *Data analysis* below) whether any fitness outcomes of hatching asynchrony were dependent on these environmental parameters.

We first extracted total monthly precipitation values from 1901-2016 at a local scale (0.5 x 0.5 degree cells) relevant for each specific population using data from the University of East Anglia's Climatic Research Unit's Time series dataset (CRU-TS 4.01, 1901-2016). This dataset reports climatic data across the globe on a 50km^2 grid, using both observations and imputed data.

For each effect size, we used mean precipitation during the breeding season to classify resource availability as being good or poor, which we used as a moderator in our meta-regressions (see *Data analysis* below for more details on meta-analyses). We focused on mean precipitation as a proxy as it has been demonstrated to be a good estimator of resource availability in studies of other ecological effects on birds, including effects on abundance and distribution (e.g., Elith et al. 2006) and life history (e.g, Mares et al. 2017). Where available, the dates for the breeding season were obtained from the relevant paper, or alternatively, estimated from the nearest population for the given species under its Handbook of the Birds of the World entry (Del Hoyo et al. 2018). We then extracted mean climatic data for the duration of the breeding season for each species at its study site.

We defined each breeding season as being good or poor, by whether it was wetter or drier than the long-term average (1901-2016) for that site. This was done by calculating the standardised rainfall anomaly (SRA), with breeding seasons classified as good when the SRA was positive, and poor when the SRA was negative. SRA is calculated as the rainfall for the particular breeding season the study was conducted in, minus the long term rainfall (for the breeding season period, but across years) divided by it's standard deviation. While it might be predicted that there may be a quadratic relationship between the SRA and breeding season quality instead of a linear relationship (i.e., extremely wet years may promote high productivity but inhibit bird foraging, e.g., Radford et al. 2001; Jahn et al. 2010), post-hoc graphical explorations of the relationship between raw SRA and our effect sizes suggested no such relationship. Five studies directly measured resource availability, used food supplementation of nests or manipulated brood sizes to create a resource limitation treatment (Bryant and Tatner 1990; Slagsvold 1986; Stouffer and Power 1991; Wiehn et al. 2000; Podlas and Richner 2013), and in these cases we used the studies' own measurement or manipulation to classify the breeding year as being good or poor. Where studies used captive populations (n = 3 across the meta-analyses, Skagen 1988; Rutkowska 2005; Mainwaring et al, 2014;), they were excluded from meta-regression models testing the dependence of the effect size on resource conditions.

We then calculated precipitation predictability for each site using Colwell's P index (Colwell 1974). Colwell's P is an information theory based index that assesses the

variation in the onset, duration and intensity of periodic phenomena, ranging from 0 (entirely unpredictable) to 1 (entirely predictable) (Colwell 1974). This index has been used in several comparative analyses investigating effects of environmental variation on variation in traits across bird species and populations (e.g., cooperative breeding; Cornwallis et al. 2017, Griesser et al. 2017, song display elaboration; Botero et al. 2009, mate choice; Botero and Rubenstein 2012).

The effect of experimental synchronisation on offspring and parental fitness may also be dependent on the extent of deviation from natural hatch spans (e.g., it might be expected that for species with naturally long hatching spans, experimental synchronisation of nests might have negative fitness consequences). Therefore, we also recorded the natural hatching span (number of days) for each species from the most geographically relevant population (either from the paper, or The Handbook of the Birds of the World, Del Hoyo et al. 2018) and included it in our models as a covariate.

Data analysis

We ran multi-level meta-analytic (MLMA) and multi-level meta-regression (MLMR) models using the R package *metafor* (Viechtbauer 2010) to evaluate support for our predictions. In all models we controlled for effect size sampling variance – weighting estimates by the inverse sampling variance (Nakagawa and Santos 2012). We took a maximum-likelihood approach to parameter estimation, accounting for study and phylogeny as random effects (Nakagawa and Santos, 2012; Chamberlain et al. 2012). However, where we had limited sample sizes (for models examining parental effort and offspring fitness, which each had 11 studies), we included only phylogeny as a random effect to avoid overfitting models.

Phylogenetic trees were constructed by taking a consensus tree of 100 samples of the pseudo-posterior species tree derived by Jetz et al. (2012), and using the Hackett et al. backbone (Hackett 2008). Phylogenetic trees were trimmed for each dataset given that different analyses used a slightly different set of taxa. In all our models we also fit an observation-level random effect to estimate a residual variance, as *metafor* does not do this by default.

We estimated the overall fitness difference between asynchronous and synchronous clutches and effect size heterogeneity using our intercept-only MLMA models that estimated study, phylogenetic and residual variances. From these variance estimates we derived, I^2 (Nakagawa and Santos 2012) for each of the levels of interest. I^2_{study} is the proportion of effect size variance resulting from between study differences, I^2_{phylo} is the proportion of effect size variance resulting from phylogenetic relatedness among taxa and I^2_{error} is the total proportion of sampling variance for each effect size (Nakagawa and Santos, 2012).

After estimating overall heterogeneity in our models, we tested the biological drivers that we *a priori* expected to moderate the effect of hatching asynchrony on offspring fitness; specifically, we included the resource availability and predictability estimates as moderators and natural hatch span as a covariate. For the model examining consequences of hatching asynchrony for offspring fitness we also included separate two way interactions between hatching order and resource availability and resource predictability (supplementary code section 2.0).

Publication bias results when studies that do not find statistically significant results are less likely to be published or when studies finding spurious effects (usually because of small sample sizes) are published earlier and thus stimulate research on a topic (Nakagawa et al. 2017). Publication bias can result from high heterogeneity among studies or even small samples sizes and so it is recommended to explore multiple graphical/statistical methods (Nakagawa et al 2017). We explored publication bias by first visually inspecting funnel plots of the residuals from the intercept only model against precision of the effect sizes (1/standard error); if publication bias exists, it is expected that a 'missing set' of effect sizes with low precision should be seen in the plots (Borenstein 2009) Second, we visually inspected for publication bias using radial (Galbraith) plots, where a zero slope indicates a lack of publication bias (Sutton 2009). We found no evidence for publication bias in any of our models (see sections 1.1.7, 1.2.7, 2.7 and 3.7 in supplementary code).

Comparative Analysis Testing Association Between Hatching Asynchrony and Environmental Predictability

Here we tested whether species/populations inhabiting environments with low resource predictability and availability have a greater hatching span. We again used precipitation as a proxy for resources, with climatic data collected as described for the meta-analyses.

Literature search and data collection

We went back over the 97 initial samples screened for inclusion into our meta-analyses. Specifically, we went over the papers excluded from this sample because they had not involved a pairwise difference between experimentally manipulated asynchronous vs. synchronous clutches. From these papers we screened the full text for a population specific estimate of hatching span and a location to enable relevant climatic data to be added (see *Collection of climatic data* below for more detail). We then conducted an additional search in Web of Science on September 29th 2018, from January 1945-September 20th 2018. The search terms were similar to our initial search ('hatch* asynchrony' OR 'asynchronous hatching' OR 'hatch* spread' OR 'hatch* span' OR 'hatch* interval') but refined by Web of Science category (include only: ECOLOGY OR ZOOLOGY OR ORNITHOLOGY OR EVOLUTIONARY BIOLOGY OR BIOLOGY). This search yielded 1742 results which were screened for broad relevance to the prediction. This yielded 120 papers whose full text were screened for a population specific estimate of hatching span and a location to enable relevant climatic data to be added. Our final sample size from this two-step approach was 75 estimates of hatching span from 67 studies, comprising 50 species (see Table S3 for list of studies included in comparative analysis).

Data analysis

We analysed the effects of resource availability and predictability on hatching span using a phylogenetic generalised mixed model implemented with the *MCMCglmm* package in R (Hadfield 2010). Prior to analysis we log transformed hatching span to better approximate a normal distribution, as it was heavily right skewed. We then ran our model, with hatching span on the log scale as the response variable, and precipitation availability and predictability as the predictors. We included phylogenetic structure as a random effect, following the same methods described above under the data analysis section for the meta-analyses. We specified the model with a moderately-weak parameter-expanded prior (V = 1, nu = 0.002, alpha.V = 1000) rather than an

entirely non-informative prior to ensure proper mixing of chains; our results were virtually identical when the model was rerun with a less informative inverse-Wishart prior (see section 4.3 of supplementary code). We ran the model with 1,000,000 iterations, 10,000 burn-in runs and a thinning interval of 100. Model diagnostics indicated good mixing of chains (Gelman 1992) and virtually no auto-correlation (lag-values < 0.1). We report the mean and 95 % credible intervals; intervals not spanning one another or zero indicate statistical significance.

RESULTS

Meta-Analyses testing fitness consequences of hatching asynchrony

Across studies, hatching asynchrony had a significant effect on individual offspring fitness (hatch order, estimate = -0.80, 95 % CIs = -1.47 to -0.13, P = 0.02). Specifically, last hatched offspring from asynchronous clutches suffered significant fitness costs compared to their last hatched counterparts from synchronous clutches (contrast Figures 1a and b). There was no evidence that these effects were dependent on an interaction between hatch order and resource availability (estimate = 1.05, 95 % CIs = -0.25 to 2.36, P = 0.11) or between hatch order and resource predictability (estimate = 5.54, 95 % CIs = -2.40 to 13.48, P = 0.17). Nor did we find any evidence that variation in effect sizes was explained by resource availability (estimate = 0.25, 95 % CIs = -0.53 to 1.04, P = 0.53), predictability (estimate = 2.27, 95 % CIs = -2.04 to 6.58, P = 0.30) or natural hatching span (estimate = 0.04, 95 % CIs = -0.15 to 0.24, P = 0.67). Finally, we found limited phylogenetic signal in our data set (0 %) with most heterogeneity in effect sizes coming from random differences between studies (I^2_{study} 78 %).

In line with the fitness results at the individual offspring level, when we examined the effects of hatching asynchrony on clutch level recruitment we found an overall negative effect size, indicating that the number of offspring surviving to fledging was greater in synchronous clutches. However, this was not significant (intercept-only model: estimate = -0.21, 95 % CIs = -0.54 to 0.13, *P* = 0.22). Unsurprisingly, there was considerable variation in effects sizes across the data set (Fig 2a, effect size heterogeneity was 54 %), with strong positive effects of experimental synchronisation on offspring survival seen in some studies/species (e.g., *Ficedula hypoleuca* (Hilstrom and Olsson 1994), *Forpus passerinus* (Stoleson and Beissinger 1997) while others reported strong negative effects

of experimental synchronisation (e.g., *Dendroica petechia* (Herbet 1993), *Parus caeruleus* (Slagsvold et al. 1994)). Neither resource availability, predictability nor natural hatching explained a significant proportion of this this variation in effect size (MLMR model: resource availability; estimate = -0.01, 95 % CIs = -0.42 to 0.41 P = 0.97, resource predictability; estimate = -0.84, 95 % CIs = -3.38 to 1.70 P = 0.52, natural hatching span; estimate = -0.07, 95 % CIs = -0.20 to 0.06, P = 0.27). Of our two random effects, phylogeny accounted for a large proportion of effect size heterogeneity (35 %), but the random study effect did not (I^2_{study} = 0 %).

These effects were qualitatively similar when we examined differences in clutch growth between experimentally synchronous and asynchronous clutches. Specifically, there was little evidence for a difference between experimentally synchronous and asynchronous clutches in overall offspring growth (estimate = -0.09, 95 % CIs = -0.36 to 0.18, *P* = 0.51, Fig 2b). Again, there was a high amount of variation in effect sizes across the dataset (Fig2b, effect size heterogeneity was 75%), with some offspring from some species/studies growing better when they hatched synchronously (e.g., *Ficedula hypoleuca* (Hilstrom and Olsson 1994), *Larus fuscus* (Bradbury and Griffith 1999)) while others grew better when they hatched asynchronously (e.g., *Parus major* (Podlas and Richner 2013)). As with offspring survival, the predictors in our MLMR model did not explain this variance (MLMR: resource availability; estimate = -0.07, 95 % CIs = -0.62 to 0.47, *P* = 0.80, resource predictability; estimate = 0.50, 95 % CIs = -3.35 to 4.36, *P* = 0.80, natural hatching span; estimate = 0.18, 95 % CIs = -0.37 to 0.73, *P* = 0.53). In contrast to the model on number of offspring fledging, phylogeny did not explain any of the variation in effect sizes (I^2_{phlyo} = 0 %). Instead, random differences between studies accounted for most of this heterogeneity (I^2_{study} = 22 %).

Finally, we examined the extent to which variation in hatching spread influenced parental effort. Overall, we found a strong trend for an effect of experimental synchronisation on parental effort. Specifically, parental effort was reduced for parents that had asynchronous broods compared to those that had synchronous broods (Fig 3). While this result failed to reach statistical significance, (intercept only model, estimate = -0.47, 95 % CIs = -1.09 to 0.14, *P* = 0.13, Fig 3) of the 19 effect sizes, only 4 (20%) reported more parental effort in asynchronous broods compared to synchronous, with the greatest effects of synchrony in parental effort observed in *Dendroica petechia*

(Herbert and Sealy 1993), *Falco sparverius* (Wiebe and Bortolotti 1995), *Falco tinnunculus* (Wiehn et al. 2001) and *Taeniopygia guttata* (Gilby et al. 2011). When expanding our intercept-only model to include moderators, we found no evidence that the variation in effect sizes was dependent on resource availability (estimate = 0.37, 95 % CIs = -0.56 to 1.29, *P* = 0.44), predictability (estimate = -1.11, 95 % CIs = -7.31 to 5.08, *P* = 0.72), or natural hatching span (estimate = 0.13, 95 % CIs = -0.17 to 0.43, *P* = 39). In contrast, a large amount of variation was explained by phylogeny (I^2_{phlyo} = 37 %) and study (I^2_{study} = 81 %).

Comparative Analysis Testing Association Between Hatching Asynchrony and Environmental Predictability

Our phylogenetic generalised mixed model used data from 67 studies, with a total of 75 data points and 50 species (see section 4.3 in supplementary code for the consensus mean phylogenetic tree of species included in this analysis) mainly distributed in Europe (Fig 4). Hatching span was 2.33 ± 1.76 (standard deviation) days on average, ranging from 0.42 to 8.6 days.

The phylogenetic signal (Pagel's Lambda, λ) of hatching asynchrony as estimated from the model was relatively strong (λ = 0.78, 95 % CIs = 0.52 to 0.98), being particularly consistent amongst passerine species (see Fig 5, lower half of the phylogeny). In line with the results from our meta-analysis, we found limited support that variation in hatching asynchrony between species was influenced by variation in our environmental moderators. This was true for both resource predictability (Fig 6, Table 1) and resource availability (Fig 7, Table 1).

Table 1: Outputs from phylogenetic generalised least squares model investigating effects of resource availability and predictability on hatching span extent. Credible intervals not spanning each other or zero indicate statistical significance.

Variable	Posterior mean	Lower 95 % Credible Interval	Upper 95 % Credible Interval
Intercept	1.39	0.52	2.29
Resource predictability	-0.87	-2.50	0.71
Resource availability	0.001	-0.002	0.005

DISCUSSION

Here, we performed for the first time, a systematic and quantitative review of the adaptive nature of hatching asynchrony in birds, testing its fitness consequences for offspring and their parents under different environmental conditions. Overall, our global study of hatching asynchrony in birds found that (1) as predicted hatching asynchrony has significant effects on individual offspring fitness – specifically last hatched offspring suffer more than first hatched offspring, (2) there is there is limited evidence that this represents a trade-off with direct estimates of female fitness (e.g. clutch level recruitment or growth), and (3) it instead appears as if there may be some benefits of hatching asynchrony for parents, in terms of reduced parental effort.

We found that hatching asynchrony had negative consequences for last hatched offspring when comparing asynchronous and synchronous clutches. Specifically, while there was no fitness difference between asynchronus and synchronous clutches for first hatched offspring, last hatched offspring in asynchronous clutches fared significantly worse than last hatched offspring in synchronous clutches. This effect was not dependent on resource availability or predictability. We then tested whether this outcome was traded off against improved clutch recruitment overall, as might be expected if the poorer success of later hatched offspring improves the survival prospects of earlier hatchings (see Mock and Forbes 1995). However, we found on difference between asynchronous and synchronous broods in terms of total clutch output or growth. Instead, we found some evidence that parents gained from hatching asynchrony in terms of reduced parental effort. Specifically, parental effort, measured in most studies as feeding rate, was lower in asynchronous compared to synchronous clutches on average across studies. This pattern matches predictions made by the peak load reduction hypothesis, which posits that hatching asynchrony benefits parents by spreading out the total food demand of the brood and negating sharp peaks in required parental provisioning (Ingram 1959). While we did not test for differences in *peak* parental effort between our two treatments, we did test mean parental effort overall, finding that it was lower in asynchronous than synchronous clutches. While this failed to reach statistical significance, 11 out of 19 effect sizes here indicated that parental effort was reduced in asynchronous compared to synchronised clutches and only 4 studies showed the opposite pattern. This result suggests that hatching asynchrony may benefit

parents in terms of saving some energy for the given breeding event to be able to then double clutch or breed again in the following year. Only one study in our meta-analysis tested the effects of experimental synchronisation on potential longer-term aspects of parental fitness. Slagsvold et al. (1994) examined differences between asynchronous and synchronised clutches on parental survival to the following breeding season, finding that there were large differences in survival between synchrony treatments, but that the direction of effect was dependent on parental sex. Further research is needed to help clarify if hatching asynchrony is common across species in functioning to help moderate parental effort and increase parental lifetime reproductive success, and to help tease out any interesting sex specific or life history dependent effects of hatching asynchrony on reduction of parental effort (Horak 1995; Stoleson and Beissinger 1995).

Across all analyses, we found no effects of resource availability or predictability on mediating the fitness consequences of hatching asynchrony or variation in the extent of hatching span across species. One explanation is that reflects that our proxies were not at a relevant scale. Most studies on the ecology of hatching asynchrony have focussed on broad environmental conditions/proxies of resources abundance and predictability that are essentially shared between individuals (weather/climate, abundance of prey, e.g., Slagsvold 1986; Temme and Charnov 1987; Amundsen and Slagsvold 1988; Pijanowski 1992; Wiebe and Bortolotti 1995). However, it has been highlighted (Amundsen 1993; Amundsen and Slagsvold 1996) that conditions may be unpredictable at smaller scales and related to the state of an individual. For example, at the start of a breeding season a female bird may be uncertain about the quality of her territory, the provisioning her mate will provide, or her nest site. Empirical studies of hatching asynchrony that incorporate these individual state related measures of predictability may be better placed to pick up brood reduction benefits of hatching asynchrony. An alternative explanation is that other factors, aside from resources, play a more important role in determining the fitness consequences of hatching asynchrony and variation in the extent of hatching asynchrony across species. Indeed, given that hatching asynchrony appears to moderate the trade-off between current and future reproduction for parents, these results generate some interesting and novel ideas about how different life history traits should influence the evolution of hatching asynchrony. For example, a prediction derived from the above is that as parents age, they should decrease hatching asynchrony

and increase parental investment, due to their decreasing residual reproductive value. In a comparative context, longer lived species may be expected to have relatively large hatching spans that moderate parental investment across years and hence increase the number of breeding attempts they have across their lifetime. These hypotheses are unexplored in the classic hatching asynchrony literature, and future longitudinal studies and comparative work that incorporates life history data across species offer an exciting opportunity to test these ideas and advance the field.

More generally, given our finding of trade-offs in fitness across family members generated by hatching asynchrony, we suggest that rather than being aligned under any of the classic hypotheses for its evolution, hatching asynchrony can be generally viewed as a mechanism mediating evolutionary conflicts of interest between parents, offspring, and siblings; parents benefit through reduced parental effort, early hatched offspring do well under both asynchronous and synchronous patterns of hatching, but later hatched offspring do poorly, particularly when hatching is asynchronous. While this pattern overall benefits parents, the extent to which it is driven by parents (by actively reduced provisioning to smaller offspring) vs. siblings (by older siblings outcompeting younger siblings or directly attacking them) is unclear (Roulin and Dreiss 2012). Studies that simultaneously measure parental effort, sibling competiton (i.e., levels of aggression or begging for parental provisioning) and fitness levels of parents and individual offspring under asynchronous vs. synchronous hatching patterns will help unravel the role of hatching asynchrony in mediating family dynamics in more detail. Combined with further studies across species that vary in ecological and life history traits, this will highlight the circumstances in which hatching asynchrony is most important for mediating these conflicts.

Overall, the combined results of our meta-analyses and phylogenetic comparative analysis in this quantitative review provide no support for the brood reduction hypothesis, the most long-standing hypothesis trying to explain the phenomenon of hatching asynchrony in birds. We did, however, find evidence suggesting that parents benefit from hatching asynchrony through reduced parental effort, but this is at the detriment to later hatched offspring. We therefore advocate for a more general and simplified perspective of hatching asynchrony, one that conceptualises hatching asynchrony as a parental effect that mediates family conflicts. Despite finding no effects

of resource availability or predictability here, future studies should explicitly examine how other life history and ecological traits affect the role of hatching asynchrony in mediating these conflicts. With this, a more detailed and clarified view of the evolution of hatching asynchrony will emerge.

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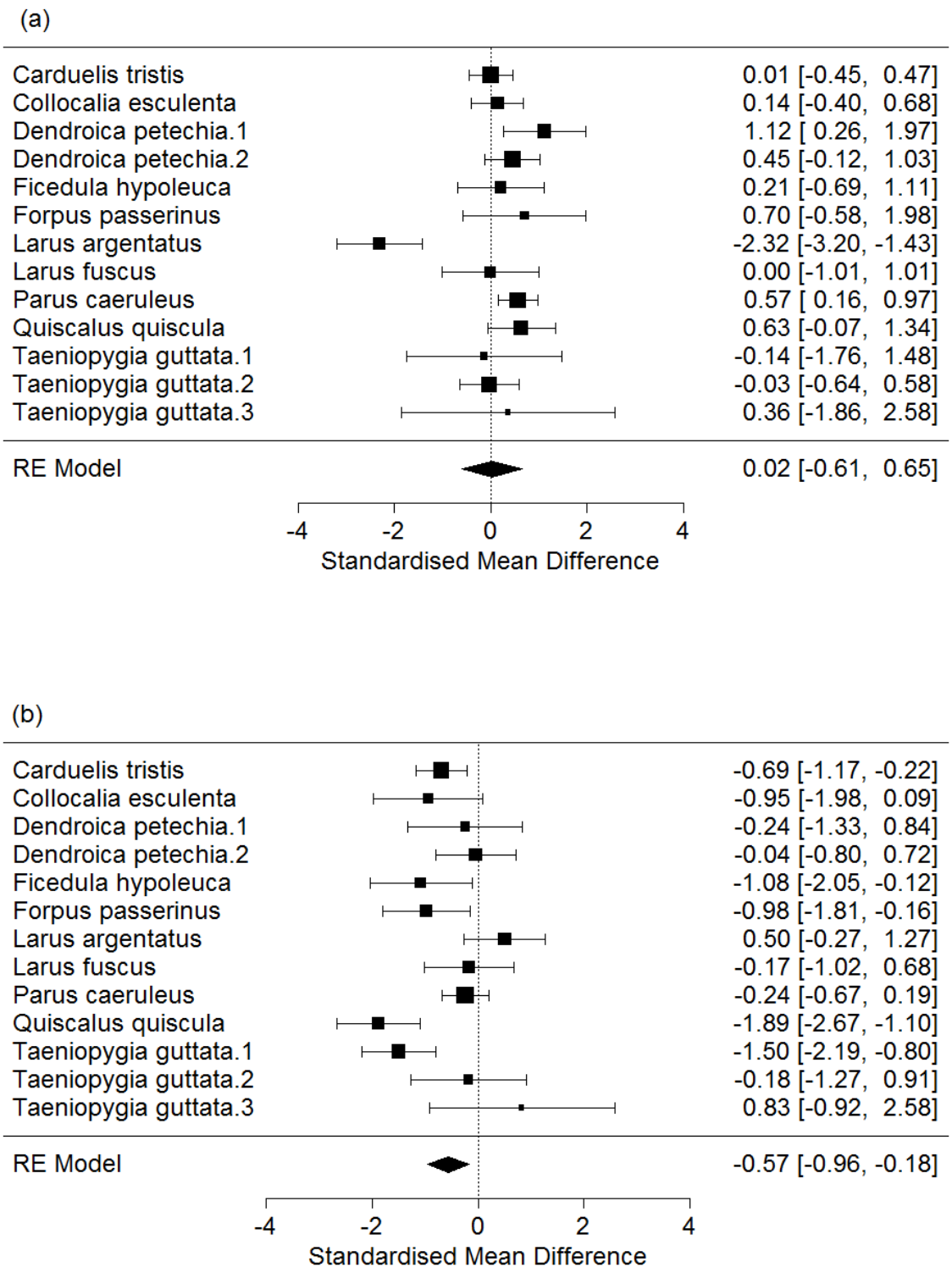

Fig. 1 Forest plot of effect sizes (standardised mean difference) and 95 % confidence intervals for studies examining differences between (a) first, and (b) last hatched offspring in asynchronous and synchronous clutches in offspring fitness. Square size for each estimate is inverse to the precision of the estimate. Where a decimal point and number is shown after a species name, this simply represents that multiple effect sizes have been taken from that species (i.e., Taenopygia guttata.3 is the fourth effect size included in the model from the species *Taenopygia guttata*).

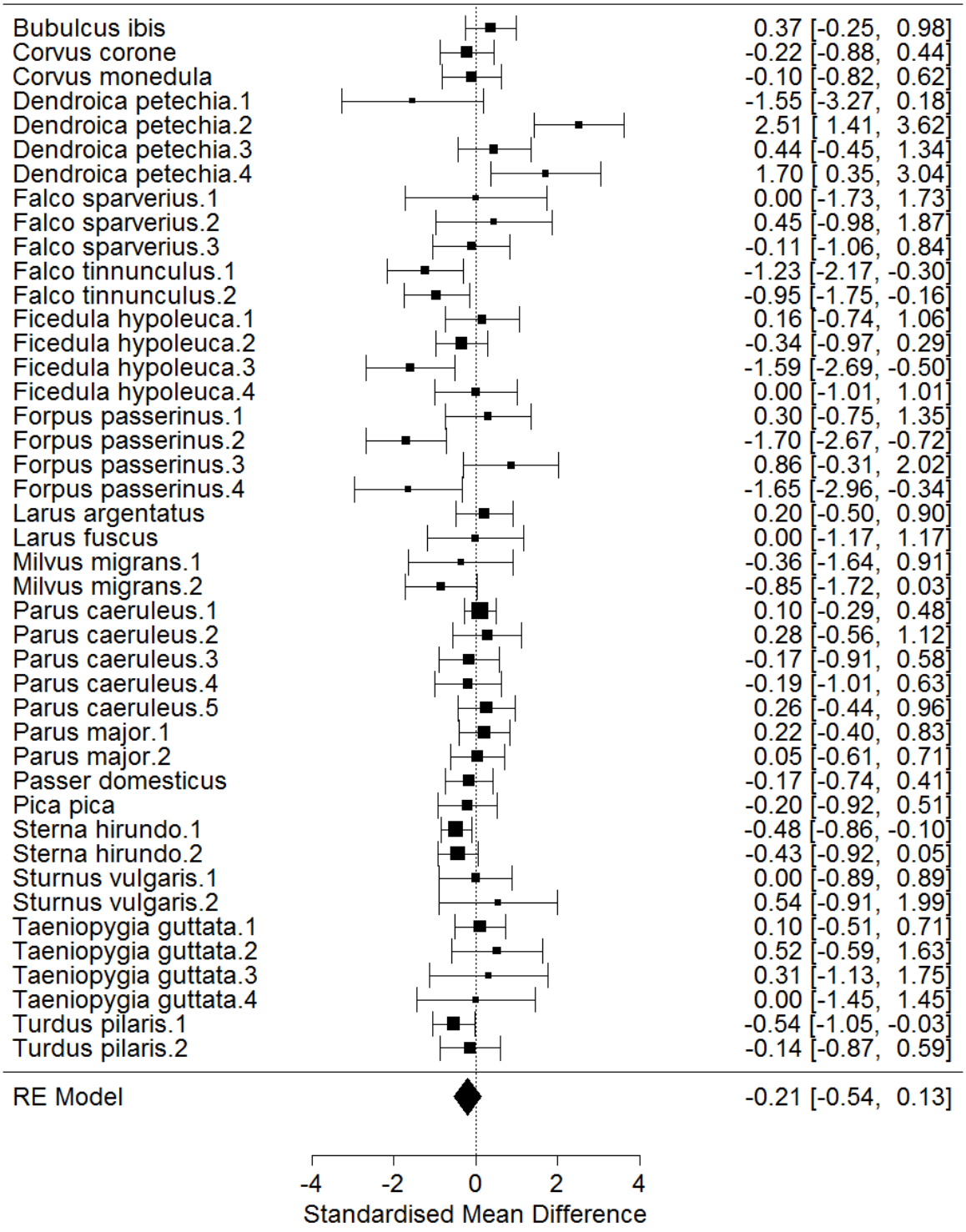

Fig. 2a Forest plot of effect sizes (standardised mean difference) and 95 % confidence intervals for studies examining differences between asynchronous and synchronous clutches in offspring survival. Square size for each estimate is inverse to the precision of the estimate. Where a decimal point and number is shown after a species name, this simply represents that multiple effect sizes have been taken from that species (i.e., Ficedula hypoleuca.4 is the fourth effect size included in the model from the species *Ficedula hypoleuca*).

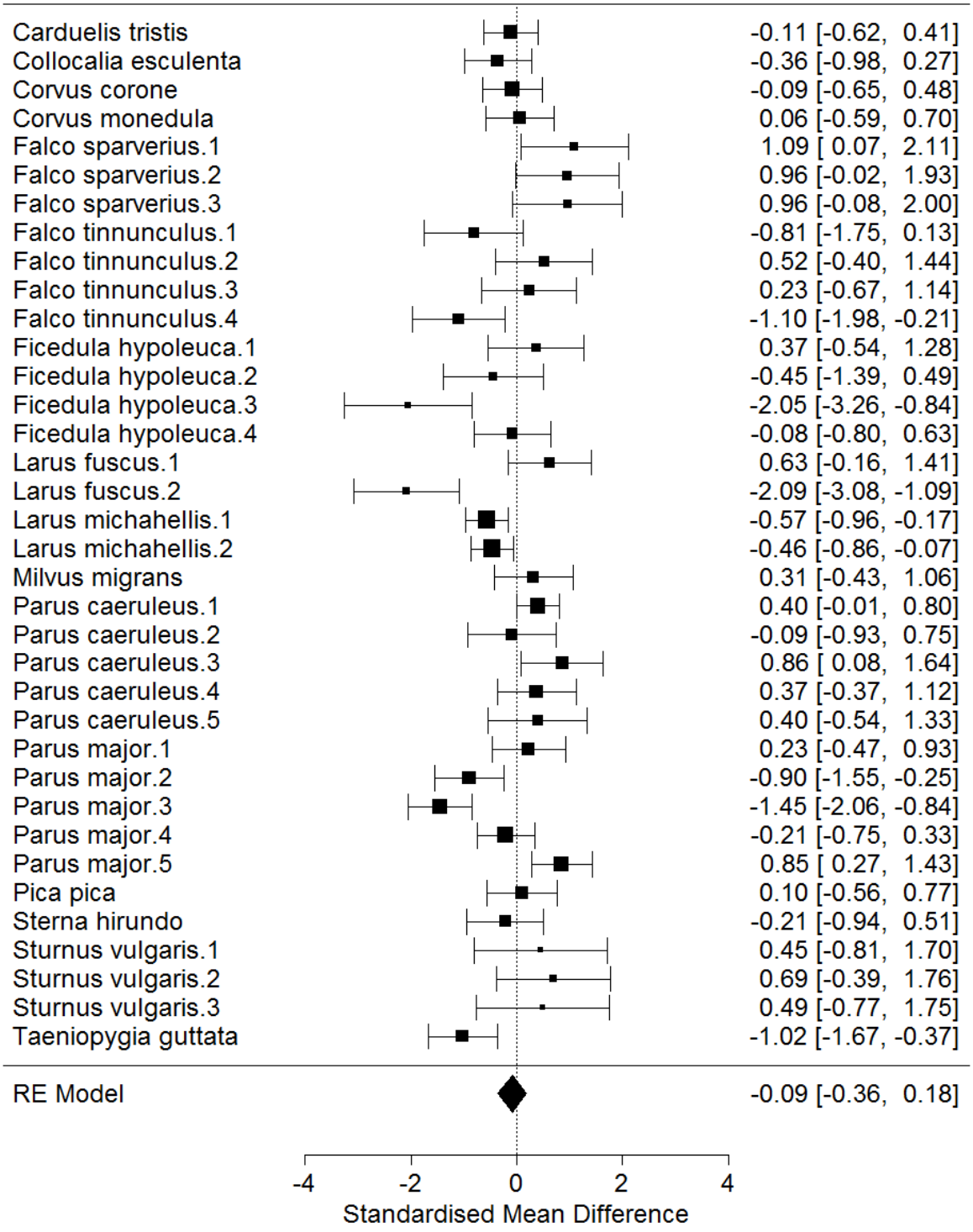

Fig. 2b Forest plot of effect sizes (standardised mean difference) and 95 % confidence intervals for studies examining differences between asynchronous and synchronous clutches in offspring growth. Square size for each estimate is inverse to the precision of the estimate. Where a decimal point and number is shown after a species name, this simply represents that multiple effect sizes have been taken from that species (i.e., Ficedula hypoleuca.4 is the fourth effect size included in the model from the species *Ficedula hypoleuca*).

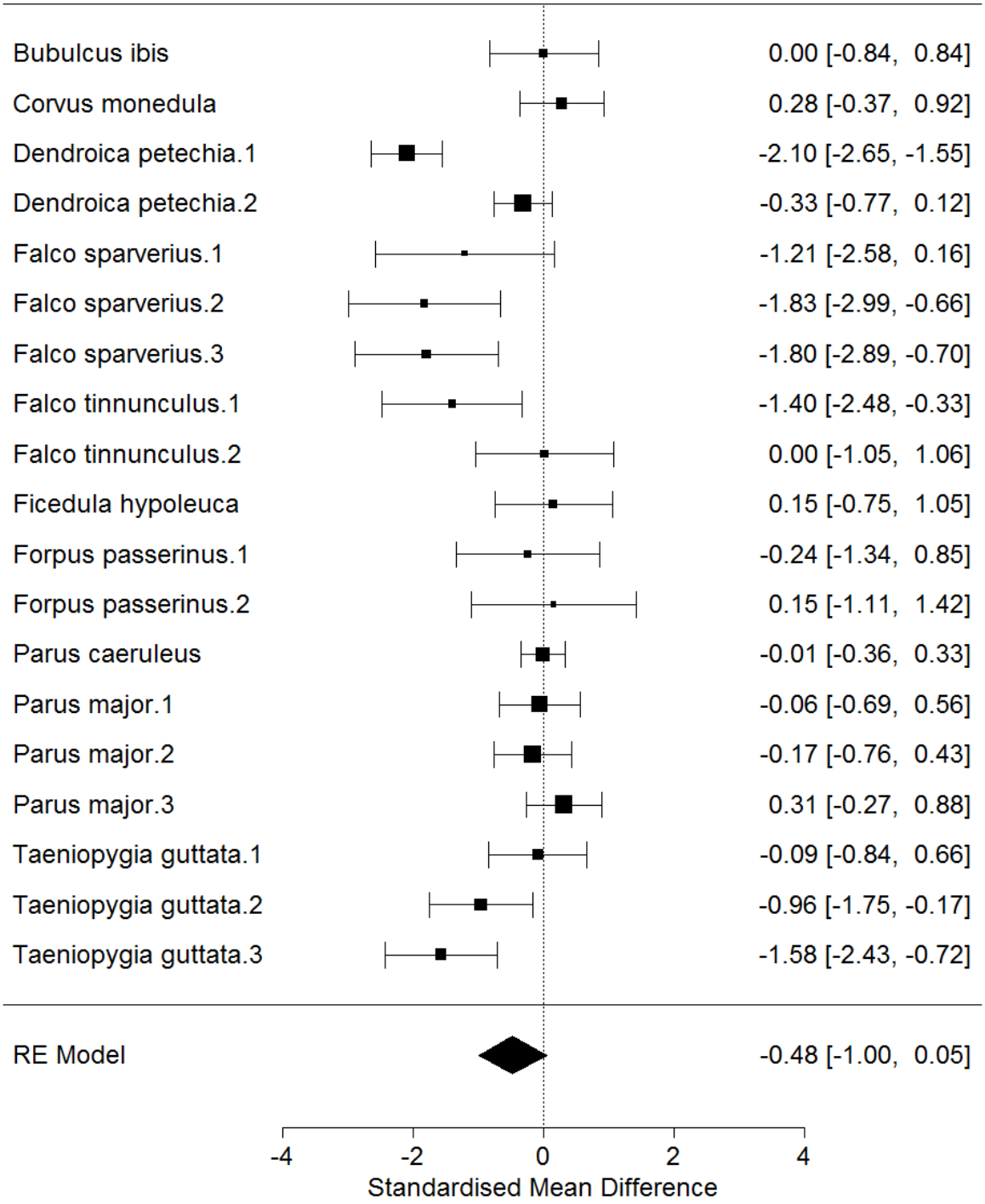

Fig. 3 Forest plot of effect sizes (standardised mean difference) and 95 % confidence intervals for studies examining differences between asynchronous and synchronous clutches in parental effort. Decimal points represent multiple effect sizes taken from the same species. Square size for each estimate is inverse to the precision of the estimate. Where a decimal point and number is shown after a species name, this simply represents that multiple effect sizes have been taken from that species (i.e., Taenopygia guttata.3 is the fourth effect size included in the model from the species *Taenopygia guttata*).

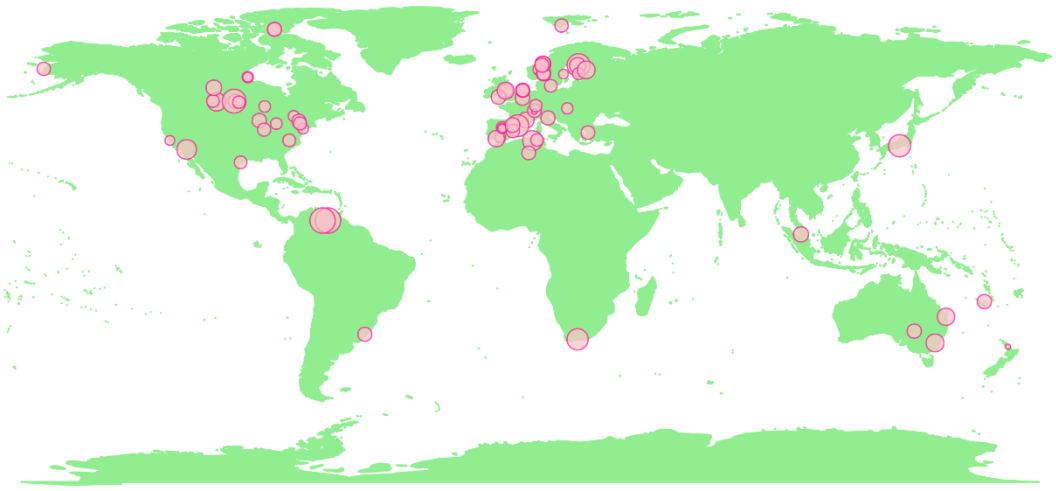

Fig 4 Distribution of populations included in comparative analysis of the effects of precipitation predictability on hatching span, with circle size representing hatching span (larger size = greater extent of hatching asynchrony).

Fig. 5 Ancestral state reconstruction of hatching span across bird species included in dataset for comparative analysis. Bars indicate extent of hatching span across the phylogeny, with each outer circle representing an extra day hatching span (range 0.42 – 8.6 days) Results were visualised using iTOL v4.4.1 (Letunic and Bork 2019).

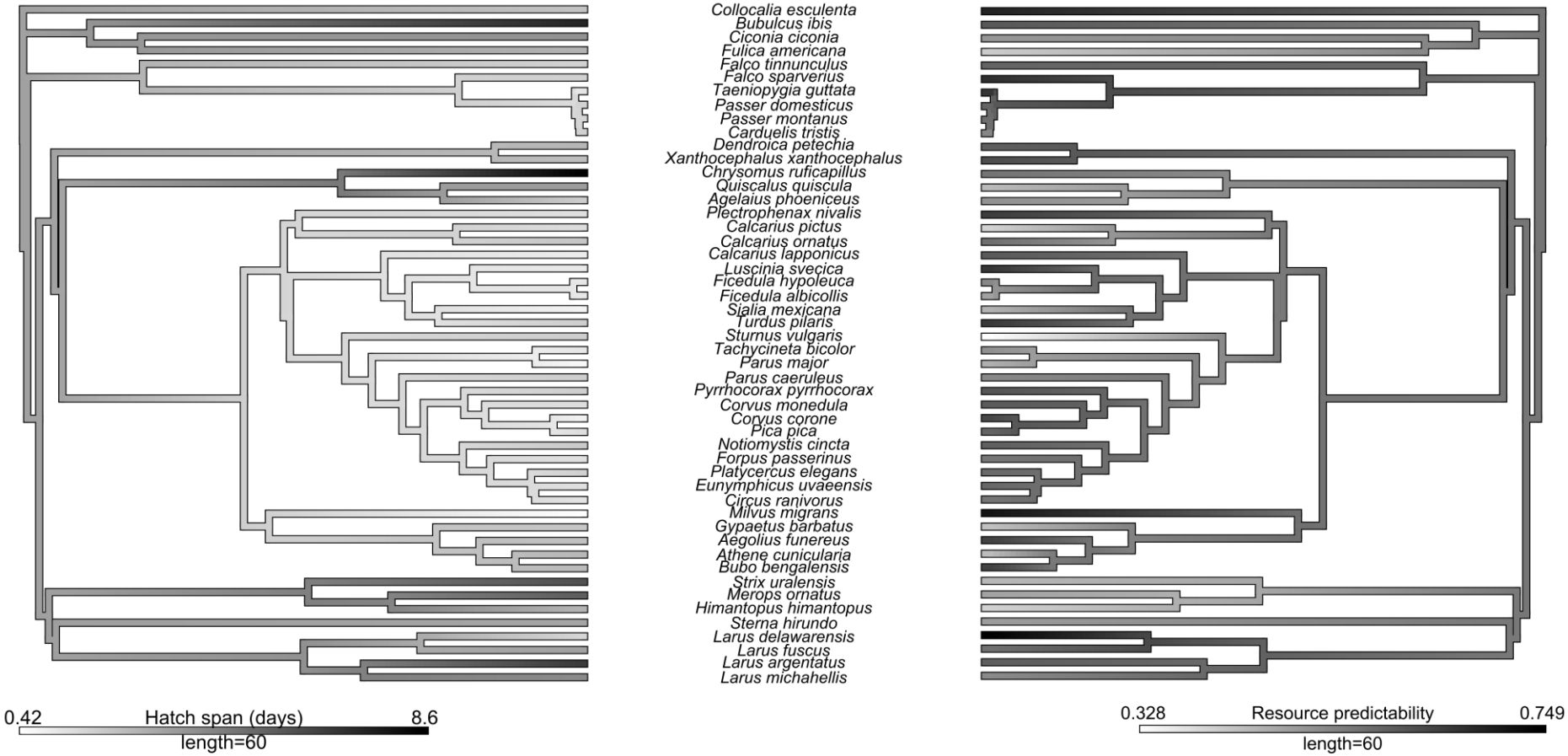

Fig. 6 Association between hatching span (left), and resource predictability (right). Predictability scores closer to 1 indicate greater predictability. Where multiple data points existed for a single species, the average values for hatching asynchrony and precipitation predictability have been taken.

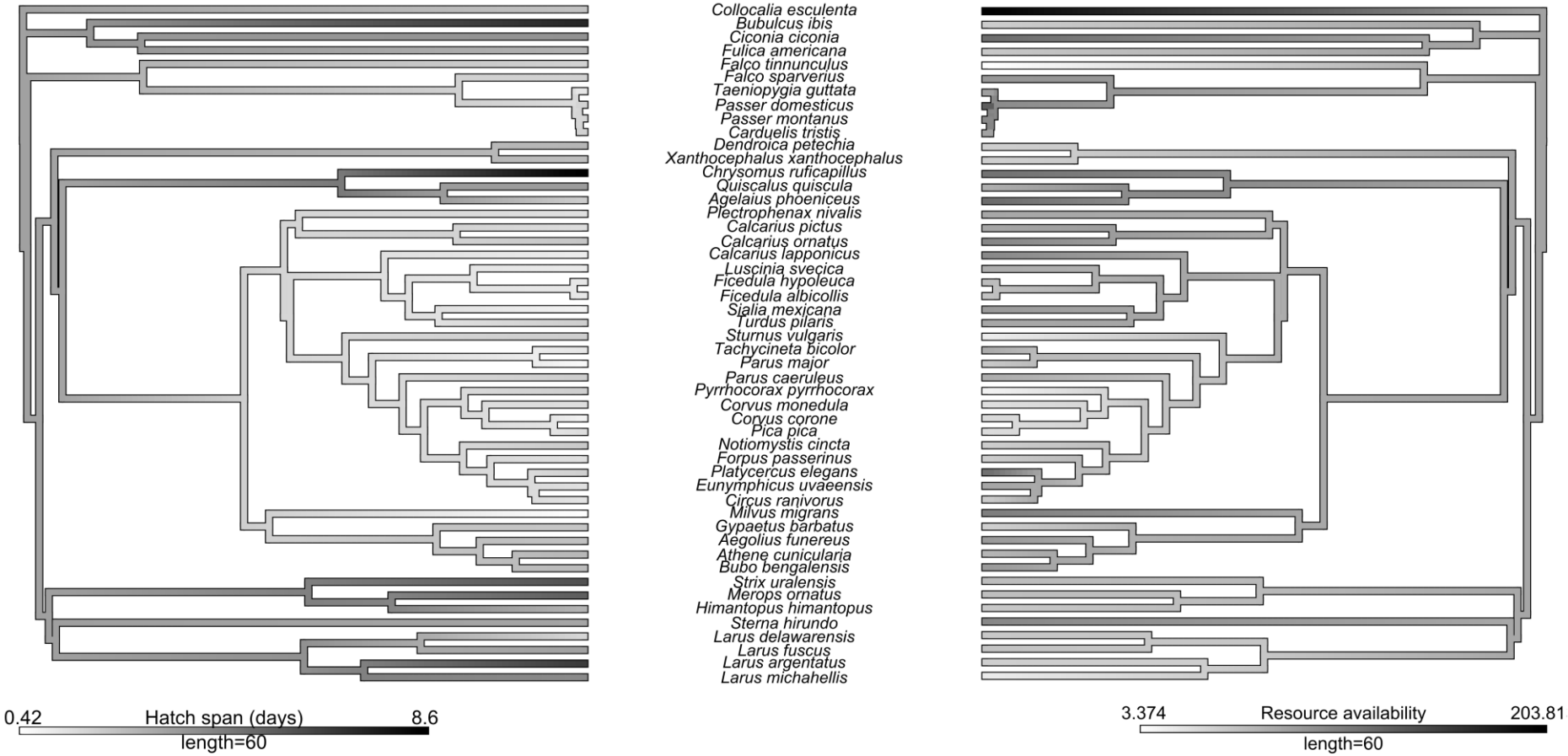

Fig. 7 Association between hatching span (left), and resource availability (right). Where multiple data points existed for a single species, the average values for hatching asynchrony and resource availability have been taken. Resource availability is represented by long term average monthly precipitation (mm).

SUPPLEMENTARY INFORMATION

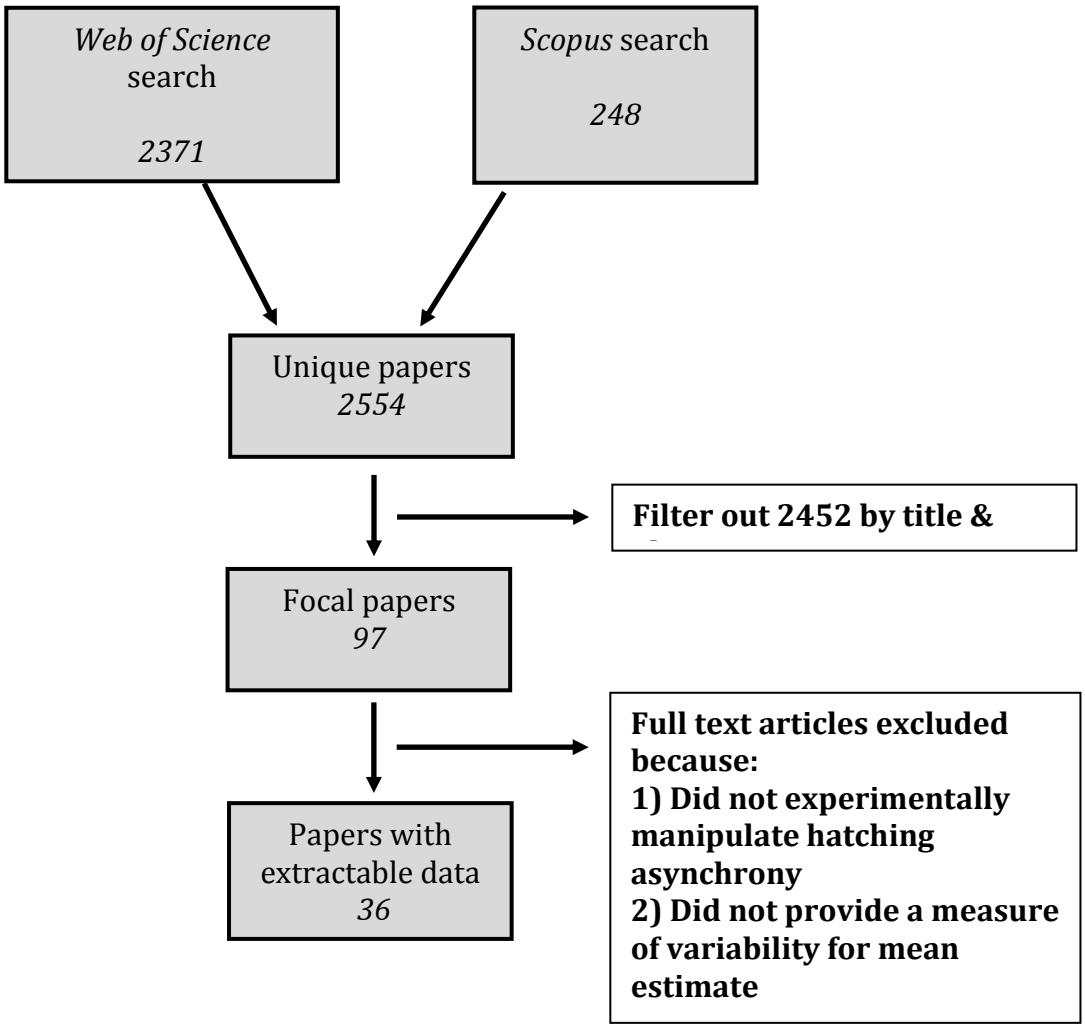

Fig S1: PRISMA flow diagram for data collection for meta-analyses.

Table S1: Fitness related traits included in meta-analyses.

Aim	Trait	Representation of trait in prediction's dataset
Does hatching asynchrony provide fitness benefits in terms of number of offspring produced?	Number of young fledged	=20/39
	% offspring survival to fledging	=15/39
	Number of recruits into breeding population	=1/39
	% of clutches experiencing brood reduction	=3/39
Does hatching asynchrony provide fitness benefits in terms of offspring growth?	Tarsus length at fledging	12/36
	Mass at fledging	24/36
Do last hatched offspring have lower fitness in asynchronous vs. synchronous clutches?	Tarsus length at fledging	6/26
	Mass at fledging	8/26
	% offspring survival to fledging	12/26
Is parental effort reduced in asynchronous compared to synchronous clutches?	Parental mass loss	1/19
	Parental metabolic rate	2/19
	Parental feeding rate (e.g., number of visits to the nest per hour or day)	13/19
	Amount of food (grams) brought to the nest per hour	2/19
	Percentage of parents surviving to following breeding season	1/19

Table S2: Initial set of studies (n = 97) identified for inclusion into meta-analyses from literature search, with reasons for exclusions.

Reference	Author/s	Year	Species	Included/Excluded with reasons
1	Aldredge	2017	*Passer domesticus*	Included
2	Amundsen and Slagsvold	1991	*Ficedula hypoleuca*	Included
3	Amundsen and Slagsvold	1991	Review article	Excluded – review article
4	Amundsen and Slagsvold	1996	Comment article	Excluded – comment article
5	Amundsen and Slagsvold	1998	*Parus major*	Included
6	Amundsen and Stokland	1988	*Phalacrocorax aristotelis*	Excluded – marine species
7	Anderson	1989	*Sula dactylatra, S. nebouxii*	Excluded – marine species
8	Arnold	2011	*Fulica americana*	Excluded – no extractable data
9	Baiao et al.	1998	*Gallus gallus domesticus*	Excluded – could not locate full text
10	Banda and Blanco	2008	*Pyrrhocorax pyrrhocorax*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
11	Barrientos et al.	2016	*Parus major, P. caeruleus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
12	Barrionuevo and Frere	2017	*Spheniscus magellanicus*	Excluded – marine species
13	Benharzallah et al.	2015	*Ciconia ciconia*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
14	Bollinger et al.	1990	*Gygis alba*	Included
15	Bosman	2014	*Larus argentatus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
16	Bosman et al,	2016	*Larus argentatus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
17	Bowers et al.	2011	*Troglodytes aedon*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
18	Bradbury and Griffiths	1999	*Larus fuscus*	Included
19	Bryant and Tatner	1990	*Collocalia esculenta, Merops viridis*	Included
20	Cotton et al.	1999	*Sturnus vulgaris*	Excluded – did not provide data on comparison of asynchronous vs. synchronised clutches
21	Cucco and Malacarne	1996	*Apus pallidus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
22	Djerdali et al.	2016	*Ciconia ciconia*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
23	Forbes and Glassey	2000	*Agelaius phoeniceus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches

24	Forbes et al.	2001	*Agelaius phoeniceus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
25	Fujioka	1985	*Bubulcus ibis*	Excluded – relevant data not reported
26	Gargett	1982	*Aquila verreauxii*	Excluded – could not locate full text
27	Gauzer	1989	*Sterna sandvicensis*	Excluded – could not locate full text
28	Gibbons	1987	*Corvus monedula*	Included
29	Gibson and Williams	2017	*Sturnus vulgaris*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
30	Gilby et al.	2011	*Taeniopygia guttata*	Included
31	Giudici et al.	2017	*Phalacrocorax atriceps*	Excluded – marine species
32	Hahn	1981	*Leucophaeus atricilla*	Excluded – no measure of variability for means
33	Harper et al.	1992	*Troglodytes aedon*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
34	Harper et al.	1994	*Troglodytes aedon*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
35	Hauber	2003	*Molothrus ater*	Excluded – comparisons were of between asynchronous and parasitised broods
36	Haydock and Ligon	1986	*Corvus cryptoleucus*	Excluded – no extractable data
37	Herbert	1993	*Dendroica petechia*	Included
38	Herbert and Barclay	1986	*Larus argentatus*	Excluded – could not locate paper and relevant data
39	Herbert and Sealy	1993	*Dendroica petechia*	Included
40	Hillstrom	1999	*Ficedula hypoleuca*	Excluded – could not locate paper and relevant data
41	Hillstrom	2000	*Larus argentatus*	Included
42	Hillstrom and Olsson	1994	*Ficedula hypoleuca*	Included
43	Howe	1976	*Quiscalus quiscula*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
44	Jackson	1993	*Ploceus taeniopterus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
45	Kumar et al.	2014	*Milvus migrans govinda*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
46	LaBarbera et al.	2017	*Troglodytes aedon*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
47	MacLeod et al.	2016	*Notiomystis cincta*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
48	Maddox and Weatherhead	2008	*Quiscalus quiscula*	Included
49	Magrath	1989	*Turdus merula*	Excluded – relevant data not reported

50	Mainwaring et al.	2012	*Taeniopygia guttata*	Excluded – no fitness related traits (only hormone levels) reported
51	Mainwaring et al.	2013	*Taeniopygia guttata*	Excluded – no fitness related traits (only personality types) reported
52	Mainwaring et al.	2010	*Taeniopygia guttata*	Included
53	Mainwaring et al.	2014	*Taeniopygia guttata*	Included
54	Martinez-Padilla and Vinuela	2011	*Falco tinnunculus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
55	Merkling et al.	2014	*Rissa tridactyla*	Excluded – Sample sizes for different asynchrony treatments were not reported
56	Merkling et al.	2014	*Rissa tridactyla*	Excluded – Not included as no extractable data provided
57	Mock and Ploger	1987	*Bubulcus ibis*	Included
58	Nilsson	1995	Review	Excluded – review
59	Nilsson and Svensson	1996	*Parus palustris*	Excluded – Not included as did involve manipulation of number of young vs. old members in a brood, rather than manipulating hatching asynchrony, and did not compare these to control group of only same aged chicks
60	Osorno and Drummond	1995	*Sula nebouxii*	Excluded – marine species
61	Parejo et al.	2011	*Coracias garrulus*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
62	Podlas and Richner	2013	*Parus major*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
63	Podlas and Richner	2013	*Parus major*	Included
64	Riehl	2016	*Crotophaga major*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
65	Rodriguez and Roper	2011	*Furnarius rufus*	Excluded – could not locate paper and relevant data
66	Royle and Hamer	1998	*Larus fuscus*	Included
67	Rutkowska and Cichon	2005	*Taeniopygia guttata*	Included
68	Saino et al.	2011	*Larus michahellis*	Included
69	Seddon and Vanheezik	1991	*Spheniscus demersus*	Excluded – marine species
70	Shaw	1985	*Phalacrocorax atriceps*	Excluded – marine species
71	Siegel et al. 1999	1999	*Forpus passerinus*	Included
72	Skagen	1987	*Carduelis tristis*	Included
73	Skagen	1988	*Taeniopygia guttata*	Included
74	Slagsvold	1982	*Turdus pilaris*	Included
75	Slagsvold	1986	*Ficedula hypoleuca*	Included

76	Slagsvold	1997	*Turdus migratorius*	Excluded – Not included as manipulated spread along a continuous scale and measured regressions
77	Slagsvold et al.	1994	*Parus caeruleus*	Included
78	Slagsvold et al.	1995	*Parus caeruleus*	Included
79	Slagsvold et al.	1992	*Corvus corone, Pica pica*	Included
80	Slagsovld and Wiebe	2007	*Ficedula hypoleuca*	Excluded – no fitness related traits reported
81	Soma et al.	2007	*Lonchura striata*	Excluded – did not include a synchronous clutch for comparison
82	Stier et al.	2015	*Parus major*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
83	Stoleson and Beissenger	1997	*Forpus passerinus*	Included
84	Stouffer and Power	1991	*Sturnus vulgaris*	Included
85	Szollosi et al.	2007	*Ficedula albicollis*	Excluded – did not contain a comparison of asynchronous vs. synchronised clutches
86	Tilgar and Mand	2006	*Parus major*	Excluded – did not contain extractable data
87	Vanheezik and Seddon	1991	*Spheniscus demersus*	Excluded – marine species
88	Vanheezik and Seddon	1996	*Spheniscus demersus*	Excluded – marine species
89	Vedder	2012	*Parus caeruleus*	Excluded – no fitness related traits reported
90	Vinuela	1999	*Milvus migrans*	Included
91	Vinuela	2000	*Milvus migrans*	Included
92	Werschkul	1979	*Egretta caerulea*	Nestling mortality and the adaptive significance of early locomotion in the Little Blue Heron
93	Wiebe	1995	*Falco sparverius*	Excluded – non-empirical study
94	Wiebe and Bortolotti	1994	*Falco sparverius*	Included
95	Wiebe and Bortolotti	1995	*Falco sparverius*	Included
96	Wiehn et al.	2000	*Falco tinnunculus*	Included
97	Zykova et al.	1986	*Larus genei*	Excluded – could not locate paper and relevant data

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Table S3: Studies (n = 67) included in comparative analysis.

Reference	Authors	Year	Species	Hatch span (days)
1	Adamou et al.	2009	*Himantopus himantopus*	1.84
2	Aldredge	2017	*Passer domesticus*	1.5
3	Alvarez and Barba	2014	*Parus major*	1.75
4	Amundsen and Slagsvold	1991	*Ficedula hypoleuca*	1.25
5	Amundsen and Slagsvold	1998	*Parus major*	1.7
6	Ardia et al.	2009	*Tachycineta bicolor*	1.61
7	Arnold	2011	*Fulica americana*	7.4
8	Banda and Blanco	2008	*Pyrrhocorax pyrrhocorax*	2.23
9	Benharzallah et al.	2015	*Ciconia ciconia*	4.7
10	Boland	2004	*Merops ornatus*	3.3
11	Bollinger et al.	1990	*Sterna hirundo*	1.9
12	Bosman	2014	*Larus argentatus*	2.06
13	Boudeffa et al	2014	*Ficedula hypoleuca*	1.48
14	Bradbury and Griffith	1999	*Larus fuscus*	2.14
15	Bryant and Tatner	1990	*Collocalia esculenta*	2.35
16	Chardine and Morris	1982	*Larus delawarensis*	1.06
17	Cirne and Lopez-Iborra	2005	*Chrysomus ruficapillus*	1.92
18	Clotfelter et al.	2000	*Tachycineta bicolor*	1.18
19	Conway et al.	2012	*Athene cunicularia*	4.5
20	de Castaneda et al	2012	*Ficedula hypoleuca*	1.25
21	Ferree et al.	2010	*Sialia mexicana*	0.8
22	Forbes et al.	2001	*Agelaius phoeniceus*	1.3
23	Forbes et al.	2002	*Xanthocephalus xanthocephalus*	1.41
24	Fujioka	1984	*Bubulcus ibis*	6.3
25	Gibbons	1987	*Corvus monedula*	2.7
26	Hebert	1993	*Dendroica petechia*	2.1
27	Hillstrom and Olsson	1994	*Ficedula hypoleuca*	0.8
28	Hillstrom et al.	2000	*Larus argentatus*	1.3
29	Howe	1976	*Quiscalus quiscula*	1.17
30	Hussell	1972	*Calcarius lapponicus*	1.7
30	Hussell	1972	*Calcarius lapponicus*	1.61
30	Hussell	1972	*Calcarius lapponicus*	1.12
30	Hussell	1972	*Calcarius ornatus*	1.49
30	Hussell	1972	*Calcarius pictus*	0.83
30	Hussell	1972	*Plectrophenax nivalis*	1.96
31	Kontiainen et al.	2010	*Strix uralensis*	3.44
32	Krebs	1999	*Platycercus elegans*	3.6
33	MacLeod et al.	2016	*Notiomystis cincta*	0.42

34	MacRoberts and MacRoberts	1972	*Larus argentatus*	1.6
34	MacRoberts and MacRoberts	1972	*Larus fuscus*	1.6
35	Maddox and Weatherhead	2008	*Quiscalus quiscula*	1.65
36	Mainwaring and Hartley	2016	*Parus caeruleus*	3.11
37	Mainwaring et al.	2010	*Taeniopygia guttata*	2
38	Margalida et al.	2004	*Gypaetus barbatus*	6
39	Mock and Ploger	1987	*Bubulcus ibis*	1.5
40	Muck and Nager	2006	*Larus fuscus*	2.03
41	Nilsson and Svensson	1993	*Parus caeruleus*	1.48
42	Pande et al.	2011	*Bubo bengalensis*	1.7
43	Podlas and Richner	2013	*Parus major*	1.83
44	Podlas and Richner	2013	*Parus major*	0.42
45	Robinet et al.	1999	*Eunymphicus uvaeensis*	2
46	Royle and Hamer	1998	*Larus fuscus*	1.84
47	Saino et al.	2011	*Larus michahellis*	2
48	Siegel et al.	1999	*Forpus passerinus*	8.6
49	Simmons	1994	*Circus ranivorus*	5.6
50	Skagen	1987	*Carduelis tristis*	2
51	Slagsvold	1982	*Turdus pilaris*	2
52	Slagsvold	1986	*Ficedula hypoleuca*	1.25
53	Slagsvold et al.	1992	*Corvus corone*	2.6
53	Slagsvold et al.	1992	*Pica pica*	2.4
54	Slagsvold et al.	1995	*Parus caeruleus*	2.1
55	Smiseth and Amundsen	2002	*Luscinia svecica*	1.1
56	Stier et al.	2015	*Parus major*	1.42
57	Stoleson and Beissenger	1997	*Forpus passerinus*	8.6
58	Stouffer and Power	1991	*Sturnus vulgaris*	1
59	Szollosi	2007	*Ficedula albicollis*	1.12
60	Theofanellis et al.	2008	*Parus major*	1.79
61	Tortosa and Redondo	1992	*Ciconia ciconia*	1.03
62	Valkama	2002	*Aegolius funereus*	6.73
63	Veiga	1990	*Passer domesticus*	0.89
63	Veiga	1990	*Passer montanus*	0.61
64	Vinuela	1999	*Milvus migrans*	2.84
65	Wellicome	2005	*Athene cunicularia*	3.8
66	Wiebe and Bortolotti	1995	*Falco sparverius*	2.5
67	Wiehn et al.	2000	*Falco tinnunculus*	2.75

Table S3 References

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CHAPTER SEVEN

General Discussion

This thesis took an integrated approach, using a combination of experimental and meta-analytical approaches to ask; how is conflict in simple and facultative family groups influenced by both relatedness and resource availability? I examined not just parent-offspring conflict as has typically been the case in this area of research (Costa 2018; Kramer and Meunier 2018) but conflict between all members of the family group. Furthermore, I examined how this conflict is mediated directly, through aggressive interactions between family members, but also indirectly, by parental effects. I did this across a range of taxa, from facultative family living lizards – the primary focus of this thesis – parental care giving beetles, to a wide range of bird species. Combined, I argue that these results have provided fundamental insights into the nature of conflict in facultative social systems and which factors may or may not be important for the stabilisation of family life early in its evolution.

In this discussion, instead of merely providing a summary and recapitulation of my findings, I aim to place these findings within a broader framework for understanding how social groups evolve (see Figure 1). In doing so, I will go beyond the specific focus of my thesis, and the main focus of much of contemporary social evolution research, which has typically focused only on why social traits such as parental care and cooperation do not break down in the face of cheating (i.e. the maintenance of social organisation via kin selection). Instead, I first focus on how kin groups initially come about (i.e., the origins of kin groups, red box in Figure 1), before discussing how these simple kin groups can be consolidated (including through kin selection, black box, Figure 1) to form the basis from which more complex forms of social organisation can emerge (blue box, Figure 1). I then go on to highlight potential avenues of future research that extend beyond the scope of this thesis, are yet relatively unexplored, and offer excellent opportunities to gain novel insights into the evolutionary causes and consequences of complex sociality.

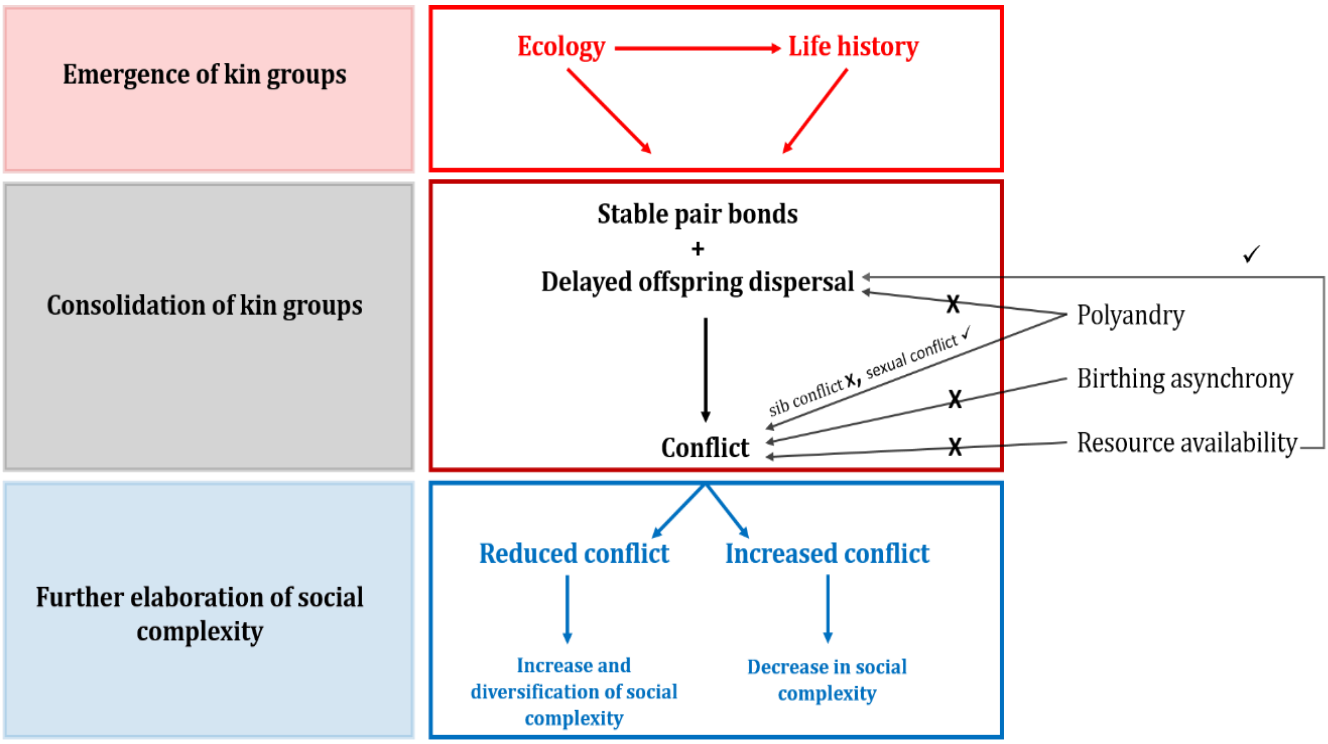

Figure 1: Diagram outlining summary of thesis results and integrated conceptual framework for investigating the evolution of complex family structures from non-social origins. (1) The red box represents the research area of understanding the ***emergence of kin groups*** from non-social origins, (2) The black box represents the research area of understanding the ***consolidation of research groups.*** Results from thesis are summarised here (**X** = no effect found, ✓ = effect found). (3) the blue box represents the research area of understanding the ***further elaboration of social complexity.***

The emergence of kin groups

The central tenant of social evolution theory is kin selection. That is, cooperative groups (and the cooperative behaviours that underpin them) will occur when the benefits of interacting with other individuals outweigh the costs, depending on the level of relatedness. However, recent research has highlighted that the evolution of complex societies cannot be understood solely from understanding the (kin) selective advantages of cooperation itself (Sterelny et al. 2014; Uller and Helantera 2014). Instead, the first step in understanding the evolution of kin-based groups, including the complex sociality exhibited by cooperative breeders and eusocial groups, is to identify the conditions promoting kin associations themselves (Figure 1). These simple kin associations then provide the opportunity for kin selection to operate and through this, for more complex

behaviours and forms of social organisation to arise (Queller 1994; Field and Brace 2004; Gardner and Smiseth 2011). Therefore, fundamental to understanding the emergence of complex cooperative societies is to first understand what brings individuals together. Only then can we understand how kin selection has acted to maintain those associations.

The defining feature of fraternal societies is an increased level of association between parents and offspring. This suggests that delayed dispersal and parental tolerance of offspring are important early steps towards the emergence of more stable and complex social organisation (Queller 1994; Field and Brace 2004; Lion and van Baalen 2008; Klug et al. 2012). Identifying the factors that help create greater association between parents and offspring can therefore help develop our understanding of the origins of complex societies. Two key traits have been suggested to be particularly important in this context; life history and ecology. Any life history trait that increases the likelihood of offspring and parent association at birth will be crucial in the emergence of kin groups. For example, the emergence of kin groups in lizards and snakes is thought to be made more likely by viviparity (live-birth) (Davis et al. 2011; Halliwell et al. 2017a). One explanation for this, is that viviparity increases the likelihood of offspring and parent association in the period shortly after birth, facilitating the evolution of basic kin groups (mothers and their offspring). Ecological factors will also be important in this context (both in their own right and in combination with life history factors, Figure 1) (Wilson 1975; Stearns 1976; Tallamy and Wood 1986; Clutton-Brock 1991; Hatchwell and Komdeur 2000; Covas and Griesser 2007; Klug et al. 2012). For example, habitat structure, quality and availability can significantly influence the movements and territories of both parents and offspring, and hence variation in these factors can determine whether parent and offspring actually associate with one another (Wilson 1975; Tallamy and Wood 1986; Lion and van Baalen 2008). Indeed, ecological constraints have been a central component of behavioural ecological studies into the evolution of delayed dispersal and cooperative breeding in birds (Emlen and Oring 1977; Komdeur 1992).

Facultative social species, such as members of the *Egernia* group, should play a powerful role in further elucidating the mechanisms underpinning the emergence of different levels of social complexity. First, they exhibit considerable variation in life history traits.

Second, they are highly amenable to experimental manipulation, allowing us to test whether key components of the environment actually elicit the emergence of kin based associations. Indeed, recent manipulative mesocosm studies in my study species, *Liopholis whitii*, have highlighted how important different components of habitat can be for the expression of parent-offspring associations. For example, Botterill-James et al. (2016) found that the layout of habitat is important; specifically, spatial aggregation of quality shelter sites resulted in more overlap between parental territories with those of their offspring. Halliwell et al. (2017b) showed that habitat saturation promotes delayed offspring dispersal and parent-offspring association, in an analogous fashion to how reduced abundance of quality breeding territories can promote retention of helpers at the nest in cooperatively breeding birds (Hatchwell and Komdeur 2000; Covas and Griesser 2007). In this thesis, I found that increased food availability (i.e., increased habitat quality) led to more delayed dispersal and parent-offspring association, presumably because of changing the direct benefits of philopatry vs. dispersing (chapter four). Outside of *L. whitii*, habitat features have been shown to be important in the emergence of kin groups (Lion and van Baalen 2008; Lancaster et al. 2011; Klug et al. 2012), although most studies in this area have used correlative evidence or modelling approaches; by using explicit manipulative approaches, future studies may be able to provide clearer links between particular ecological factors, patterns of movement by parents and offspring, and the emergence of sociality.

Once life history or ecological factors bring parents and offspring together, if the costs of tolerating offspring are low enough for parents and the benefits of associating with parents versus dispersing are high enough for offspring (for example, through enhanced social learning opportunities and cognitive development, Munch et al. 2018a) then these kin groups will be relatively stable (Klug et al. 2012). Any pre-existing behaviours that function outside of a social context may also be important to this initial emergence of kin groups (Tallamy and Wood 1986; Field and Brace 2004; Moore and Benowtiz 2019). For example, aggressive territoriality or anti-predator defences can easily be co-opted into behaviours that become reinforced through kin selection (for example, to function in defence of eggs or live offspring, see Huang and Pike 2011, 2013, Huang et al. 2013). The next step in the framework – such as the studies in this thesis (chapters two, four, five,

six) – is to identify how different factors reinforce these social associations and behaviours and promote the consolidation of these basic kin groups.

The consolidation of kin groups

Once kin groups are formed, a new social environment is created where parents and their offspring are aggregated and interactions between family members are intensified. The next step towards complex sociality is for these kin groups to be consolidated via repression of conflict and the promotion of cooperation in the new social environment (Bourke 2011, Figure 1). This was the main focus of my thesis, and I include a discussion of my results within this context in this section.

Kin selection has classically been invoked to explain how conflict is minimised in kin groups; high relatedness between kin enables indirect fitness benefits of helping and associating with kin (or vice-versa, conflict with kin induces indirect fitness costs, reducing negative interactions between kin), and so kin groups can be consolidated (West et al. 2015). Therefore, any factor that increases the level of within group relatedness should be fundamental to the stabilisation of kin groups. This can occur when rates of extra-pair mating (promiscuity) are low. This has been shown to be crucial for transitions to and the maintenance of complex kin groups. For example, cooperative breeding in both birds and mammals is enabled by low promiscuity (Cornwallis et al. 2010; Lukas and Clutton-Brock 2012) and only full sibling families in insects have evolved eusociality (Hughes et al. 2008). In contrast, the dissolution of cooperative breeding to less complex social systems has occurred primarily in lineages where promiscuity was high (Cornwallis et al. 2010).

While the effects of relatedness on complex cooperative behaviours are well supported, the role that relatedness plays in the consolidation of simple family groups has been less well explored (but see While et al. 2009; Falk et al. 2014; Ruch et al. 2014a, b). In this thesis I empirically tested how promiscuity affects kin interactions and stability of kin groups in a simple and facultatively family living lizard species. Interestingly, I found no major effect of promiscuity on either sibling conflict or changes in family structure via offspring dispersal (chapter four). This contrasts with previous research in this and related species that showed that extra-pair mating can affect family structure via paternal aggression towards non-kin offspring (Bull et al. 2000; While et al. 2009). This

could possibly be because promiscuity results in relatedness coefficeinet of 0 between fathers and extra-pair offspring, whereas extra-pair offspring still have a relatedness coefficient of 0.25 with their siblings, resulting in inclusive fitness costs from sibling conflict but not father-(extra-pair)-offspring conflict. Like my findings in *L. whitii*, I found no effect of promiscuity on sibling conflict in another facultatively family living species, the burying beetle *Nicrophorus vespilloides* (chapter five). These findings contrast with recent studies in other simple and facultative social systems that have demonstrated effects of relatedness between siblings on their interactions (e.g., earwigs, Dobler and Kolliker 2011; Falk et al. 2014, spiders, Ruch et al. 2014a, b). This thesis therefore suggests a need for more empirical studies in other simple and facultative social systems to identify the particular circumstances in which relatedness between siblings is important for their interactions and consequent stability of the groups that they form.

A second factor that should be important for conflict repression and consolidation of kin groups are the costs versus benefits of helping kin. If the direct costs of helping kin (in terms of the reproductive cost for the helper) outweigh the indirect benefits (in terms of the number offspring produced by the recipient of the help), then conflict will be selected for over cooperation (Hamilton 1964) and the stability of kin groups will be compromised. This idea has been particularly well studied in cooperatively breeding bird species, (reviewed in Emlen 1997; Dickinson and Hatchwell 2004) and has received some support. For example, in the Seychelles warbler (*Acrocephalus sechellensis*), helpers aiding in raising their full siblings do not work as hard their parents (Komdeur 1994), even though both should gain the same indirect fitness benefit from helping (i.e., r = 0.5 for both helpers and parents). This variation can be explained by the costs of helping; as residual reproductive value is higher for the helpers, the cost to benefit ratio of helping is higher and so they invest less into helping (Downing et al. 2015). In the context of simple family groups characterised by relatively simple social interactions that represent the early stages of the evolution of complex sociality, it can be conceptualised that if the costs of tolerating kin outweigh the benefits, then conflict between kin will proliferate and family structures will revert to solitary living). In my thesis, using a lizard that exhibits simple and facultative sociality, I manipulated food availability – which mediates the relative costs versus benefits of tolerating kin (Mock et

al. 1987; West et al. 2002; Bourke 2014) – and measured effects on sibling conflict and consequences for family group maintenance in terms of whether offspring remained or dispersed from their family group (chapter four). I found that when food availability was high (i.e. the costs vs. benefits of tolerating kin were low), offspring delayed dispersal much more. However, there was no concomitant effect on sibling conflict, suggesting that the effect of food availability on family structure was mediated by direct rather than indirect fitness effects for offspring (chapter four). Studies such as this are rare relative to those on the costs and benefits of helping in more advanced systems, so further research is needed to determine if the costs and benefits of cooperation (or simply kin tolerance) are as important as they are in advanced social systems and as predicted by kin selection theory.

The effects of kin selection on conflicts within family groups can be mediated directly, or in more complex ways, such as through parental effects (Smiseth and Royle 2018). For example, parents can mediate sibling conflict and parent-offspring conflict through a myriad of differential allocation processes, for example through differential distribution of resources (such as hormones) to offspring before birth or at laying (Muller et al. 2007; Smiseth et al. 2011) and mothers may manipulate paternal care via pheromones (Engel et al. 2016; Paquet and Smiseth 2016; Paquet and Smiseth 2017; Smiseth and Royle 2018). Indeed, in the case of sibling conflict, differential resource allocation has been shown to have strong effects on levels of aggression and competition at both the brood (Smiseth et al. 2011) and the individual level (Duckworth et al. 2015).

Another parental effect that could mediate sibling conflict is the creation of developmental asymmetries in offspring, achieved by spreading out the birth of offspring (Roulin and Dreiss 2012). I explored this idea in my thesis, in the context of the effects of birthing asynchrony in *L. whitii* (chapter four), and hatching asynchrony in bird species (chapter 6). Despite large variation between *Liopholis whitii* mothers in the extent of birthing asynchrony (0 -7 days), birthing asynchrony had no effect on the extent of sibling conflict. This result is consistent with previous work finding no effect of birth order and spread on offspring dispersal behaviour (Halliwell et al. 2017b). Combined, these results suggest that birthing asynchrony may have a limited role in mediating family conflict and structure, However, such an effect cannot be entirely ruled out. The above studies have only been able to test how temporal staggering of offspring

production affects family conflict and structure; an unexplored idea is that birthing asynchrony may influence family conflict and structure through the spatial spreading out of offspring. Mothers may give birth to a core group of offspring within her and her partner's territory, but then move away to give birth to extra-pair offspring who would otherwise be attacked and even killed by their social (non-genetic) father (While et al. 2009). This is a strategy that mothers could use to offset the costs of undertaking extra-pair mating. This is an exciting and novel hypothesis for how family conflict is mediated in *L. whitii* kin groups and could be tested by expanding from the experimental enclosure approaches used in this thesis (chapter four) and Halliwell et al. (2017b) into a field-based setting.

Hatching asynchrony was important in determining the outcome of parent-offspring conflict across bird species (chapter six). Specifically, I used a set of meta-analyses to show that hatching asynchrony helps parents reduce their parental effort, but that this negatively affects the survival and growth of last hatched offspring. This chapter highlights how family conflicts can be mediated by nuanced mechanisms and cannot be understood purely from studying the conspicuous behavioural conflicts that occur in families. Studies of the role of parental effects on family dynamics have been relatively well explored in the context of hatching asynchrony (Magrath 1990; Stoleson and Beissenger 1995; Stenning 1996; this thesis, chapter six) and the differential allocation of resources, such as hormones, to eggs in bird species (Muller 2007). The role of parental effects and other indirect mechanisms on family dynamics has been less well explored outside of birds, receiving particularly little attention in studies of simple and facultative social systems (but see Smiseth and Morgan 2009; Engel et al. 2016; Ford et al. 2016; Paquet and Smiseth 2017). Investigating parental effects in these less explored systems represents an excellent opportunity to build a more detailed picture of the mechanisms involved in the consolidation of families and mediation of the evolutionary conflicts of interest occuring within them.

One important caveat to make at this point is that, while the focus of my thesis was primarily on sibling conflict (chapters four and five), parent-offspring conflict (chapters three and six) and sexual conflict (chapter two), there are various interactions within kin groups – both cooperative and competitive – that can be important for the consolidation vs. dissolution of social structure. Specifically, these other interactions are

(1) sibling cooperation, (2) offspring assistance towards parents, and (3) parent-offspring competition (Kramer and Meunier 2018). These interactions have been well explored in the context of cooperatively breeding bird species where adult siblings help their parents raise junior siblings. These interactions may also be important for consolidation vs. dissolution of the precursors to these more complex kin groups (Kramer and Meunier 2018). For example, the consolidation of simple kin groups may occur when sibling cooperation provides offspring with complementary benefits to parental care. Similarly, offspring assistance towards parents may promote the consolidation by reducing the costs of parental care. Conversely, parent-offspring competition could counteract the benefits of care and promote the dissolution of kin groups. These processes have only recently started to receive empirical and theoretical attention (reviewed in Kramer and Meunier 2018), but where they have been investigated, they have been found to have important effects. For example, offspring investment into social immunity can benefit parents and their siblings (Diehl et al. 2015; Körner et al. 2016) which may promote the maintenance of kin groups (Meunier 2015; Van Meyel et al. 2018). In *Liopholis whitti*, these neglected interactions may be less important; the relatively simple behaviours that characterise family groups (involving merely tolerance of kin) likely precludes sibling cooperation or offspring assistance towards parents, and I found little evidence of parent-offspring competition (chapter four; appendix one. See also Botterill-James et al. 2016). In contrast, such interactions – particularly sibling cooperation – may play an important role in the maintenance of *Nicrophorus vespilloides* families. For example, Schrader et al. (2015) argue that sibling cooperation in carrion digestion is a key benefit of family living for *N. vespilloides* larvae, particularly in the absence of facultative parental care (but see Magneville et al. 2018 for arguments against evidence of sibling cooperation in this species). Explicit consideration of these mechanisms in future studies in a range of taxa may help in building a more detailed and accurate understanding of how kin groups are maintained (Kramer and Meunier 2018).

Kin groups are also more likely to be consolidated when there are mechanisms that enforce cooperation over conflict between individuals (Trivers 1971; Axelrod and Hamilton 1981; Frank 1995; Frank 2003; West et al. 2007; Agren et al. 2019), such that the benefits of acting in a way that enhances individual reproductive success at a cost to

kin is reduced (Frank 1995). For example, policing and punishment of anti-social behaviour has been shown to be important for the evolution of complex sociality in some eusocial Hymenopterans, where workers destroy eggs laid by other workers (Ratnieks 1988; Ratnieks and Visscher 1989; Wenseleers and Ratnieks 2006). Across species, higher effectiveness of policing reduces the number of workers reproducing, particularly when group relatedness is low (and so the potential for within group conflict is high). This enforcement strategy hence suppresses within group conflict – in terms of non-reproductive castes 'cheating' the rest of the group – and helps to prevents the collapse of complex sociality (Wenseleers and Ratnieks 2006). The role of enforcement strategies for the maintenance of cooperation vs. conflict – and the maintenance of social group cohesion vs. dissolution – has also been explored in the context of intragenomic conflict (Hurst et al. 1996), cancer suppression (Dunn et a al. 2004) and in social primates (Flack et al. 2006). The role of enforcement mechanisms in less complex kin groups, such as simple and facultative family groups, is less well explored. In this thesis I found some evidence that punishment may play an important, if indirect, role in the maintenance of simple family groups. Specifically, males punished their female breeding partners when they undertook extra-pair mating (chapter three). Males also appear to punish females that undertake extra-pair mating by not accepting non-kin offspring on their territories (While et al. 2009). These punishments may increase the costs of multiple mating for females and increase the incidence of genetic monogamy, thereby enabling kin selection to repress conflict between fathers and offspring and consolidate kin groups. This also sets the stage for the further elaboration of social complexity (see below).

Further elaboration of social complexity

Once the consolidation of simple kin groups has occurred, the next step is to understand whether these forces have a consistent effect and drive the evolution of more complex social systems and cooperative behaviours (Figure 1). There has been a swathe of empirical and theoretical work aimed at understanding how life history traits, habitat constraints, kin selection, and a combination of these forces, have driven the evolution of cooperative breeding from more simple kin groups, particularly in bird species (e.g. Emlen 1994; Cockburn 1996; Arnold and Owens 1998; Hatchwell and Komdeur 2000; Pen and Weissing 2000; Kokko et al. 2002; Covas and Griesser 2007; Griesser et al.

2017). Comparative work has provided many insights into some important drivers of complex sociality. For example, low rates of promiscuity facilitate transitions to cooperative breeding in birds (Cornwallis et al. 2010), mammals (Lukas and Clutton-Brock 2012) and eusociality in insects (Hughes et al. 2008; Boomsma 2011), although in cichlid fishes, direct benefits of cooperation appear to be more important than indirect benefits for the evolution of cooperative breeding (Dey et al. 2017). In birds, unpredictable and harsh environments also appear to promote transitions from family to cooperative living (Rubenstein and Lovette 2007; Jetz and Rubenstein 2011; Griesser et al. 2017), and life history factors such as low mortality of breeding adults have also suggested to be important (Arnold and Owens 1998; Covas and Griesser 2007; but see Blumstein and Moller 2008 for potential confounding factors). Despite these insights, there has been little advance in disentangling the relative importance of these different evolutionary forces for the emergence of complex social organisation and cooperative behaviours, both within and across different taxa. A better understanding may be developed by in two ways.

First, investigating the elaboration of social complexity in non-traditional systems may offer fresh insights into how complex sociality evolves. Most research into the evolution of complex sociality has focussed on birds, mammals and Hymenopterans (West et al. 2007), with less attention paid to species such as termites (Thorne 1997), social spiders (Jackson 2007; Pruitt and Aviles 2018), aphids (Stern and Foster 1996), and marine species generally (Wong and Balshine 2011; Hing et al. 2017). Expanding research efforts into new model taxa may help clarify the roles of different factors in the evolution of complex sociality. For example, a more detailed examination across a wider range of taxa may elucidate the relative importance of different drivers of sociality across the animal kingdom and provide insights into any context dependent effects of these drivers.

Second, stronger inferences about casual effects of different factors on the elaboration of social complexity may be made by combining comparative approaches with targeted experimental and observational work. Comparative studies have shown that ecological factors can have strong effects in promoting or constraining the evolution of complex sociality (Rubenstein and Lovette 2007; Jetz and Rubenstien 2011) and such studies have been instrumental in highlighting broad geographical patterns and ecological and

life history correlates of sociality (e.g.; Du Plessis et al. 1995; Arnold and Owens 1998; Guevara and Aviles 2015). However, these studies often fail to elucidate in detail the mechanisms through which these correlates affect sociality. For example, different ecological conditions can affect sociality through moderating either indirect or direct fitness outcomes of cooperation (or both) or are unable to disentangle the effects of a suite of covarying traits (Griesser et al. 2017; Hing et al. 2017). Moving forward, integrating comparative phylogenetic analyses with targeted observational and experimental work should help in developing a more comprehensive and detailed view of the evolution of complex sociality. In particular, such an integrated approach should allow researchers to (1) disentangle the effects of suites of correlated traits on social evolution, and (2) understand transitions in social complexity in greater mechanistic detail, by linking the effects of different causal factors on individual level facultative changes in behaviour with broad macro-evolutionary shifts in social organisation.

Future research topics

Above, I have outlined an integrated framework for conceptualising the evolution of complex kin-based sociality from non-social origins, discussed the results of this thesis within the context of this framework (particularly with reference to the consolidation of kin groups), and highlighted remaining knowledge gaps and areas for future research. I now briefly outline three emerging research topics that go beyond the scope of the studies in thes is, and which have great potential to provide novel insights into the causes and consequences of complex sociality.

Future research topic 1: Roles of kin selection, life history and ecological factors in the early evolution of social complexity

As detailed above, there has been a plethora of comparative studies investigating different drivers of complex sociality in birds, mammals and eusocial insects. Such studies have highlighted that high levels of relatedness between kin has been a crucial factor for transitions to cooperative breeding in birds and mammals (Cornwallis et al. 2010; Lukas and Clutton-Brock 2012) and eusociality in insects (Hughes et al. 2008; Boomsma 2011). There is also evidence that ecological and life-history traits can play a role in mediating transitions to cooperative breeding in birds (e.g., Arnold and Owens 1998; Covas and Griesser 2007). However, in these systems sociality is derived, obligate

and quite complex. As such, these studies can inform us how different factors have promoted the evolution and diversification of quite complex social systems from already social ancestors but cannot tell us much about the *early evolution and diversification of sociality from a non-social origin*. To understand the early evolution and diversification of family life we instead need systems where (1) social behaviour is relatively simple, (2) social life is facultative, and (3) there is variation in sociality both within and between species.

The Australasian clade of *Egernia* lizards, which my thesis focal species *Liopholis whitii* belongs to, represents an excellent study system with which to use a combined approach of comparative, observational and experimental studies to address this knowledge gap. The *Egernia* group is comprised of approximately 60 species from seven genera (Gardner et al. 2008). There is a wide range of social systems found within this group; some species live solitarily, some species form long term pair bonds without delayed offspring dispersal, and some display relatively complex social systems with extended multigenerational families containing up to 30 related individuals (reviewed in While et al. 2015; Whiting and While 2017; While et al. 2019). There is also great variation between (and often within) species in the environments they inhabit and levels of social group relatedness (Chapple 2003). Therefore, the *Egernia* system offers a feasible and powerful approach to link selection on individual behaviours with macro-evolutionary patterns of sociality, through (1) collection of more detailed ecological, life history and genetic data across species for comparative analysis, combined with (2) manipulative studies within species that examine individual level responses to variation in the parameters of Hamilton's rule and other factors (such as the studies presented in this thesis). Such approaches may find that the early evolution of family life is analogous to the evolution of highly complex systems, where kin selection is important to individual social interactions and group structure (e.g., Hughes 2008; Cornwallis et al. 2010; Lukas and Clutton-Brock 2012) or may instead reveal other factors that are important drivers of sociality.

Future research topic 2: Genetic and physiological mechanisms underpinning variation in social behaviours and structures

A complementary approach to the above is to identify the molecular and physiological mechanisms underlying broad scale, across species patterns of sociality. Such research could help determine whether shared proximate mechanisms are associated with particular social behaviours and structures across species, or if there are sets of alternative mechanisms that lead to convergence in sociality. Characterising these patterns should then advance our understanding of how sociality arises, diversifies and is facilitated or constrained by underlying mechanistic processes.

A key question to ask in this framework is: what are the genomic features underpinning the evolution of complex social behaviours and structures? As the logistics of sequencing and assembling transcriptomes – and even genomes – becomes easier, answering the above question is becoming more feasible (Rubenstein and Hoffman 2015). For example, genomic studies of eusocial Hymenoptera (Toth et al. 2007; Smith 2008) and termites (Terrapon et al. 2014) have identified genes and gene sets that are linked to specific social behaviours. There are even studies elucidating the roles of epigenetic mechanisms (Yan et al. 2014; Bewick et al. 2016; Li-Byarlay 2016; Harrison et al. 2018) and genetic regulatory networks (Bloch and Grozinger 2011) on the expression and transitions between social phenotypes, including caste differentiation in eusocial systems. These studies have proven useful, for example by highlighting that convergence in some of the changes to gene regulation and associated effects on pheromone production has underpinned the evolution of caste differentiation and transitions to eusociality in both termites and Hymenoptera (Harrison et al. 2018). There is also great potential for further insights into the evolution of complex sociality using comparative genomic approaches by shifting focus away from the Hymenoptera and into non-model systems (Taborsky et al. 2015; Kronauer and Libbrecht 2018). In particular, novel insights can be made by investigating across taxa the extent to which genetic effects on the evolution of complex sociality are the result of co-option and regulation of genes that function in other contexts or are the result of the emergence of novel genetic elements (or a combination of these two processes).

Comparative work on neurohormonal mechanisms is another exciting approach for gaining novel insights into the evolution of complex sociality, as these mechanisms seem to play an important role in a range of social behaviours across a range of taxa differing in social complexity and organisation (Kasper et al. 2017). Despite this, very little is known about links between neurohormonal processes and between species variation in sociality, as such links have only been thoroughly explored in a restricted set of mammalian species (Fischer et al. 2019). Where these links have been explored, they have produced interesting insights into key social behaviours involved in complex social organisation. For example, the neuroendocrine basis of pair bonding has been well studied in mammals and to a lesser extent, other vertebrate taxa. The oxytocin system and its homologs (for mammals; oxytocin, for birds, reptiles and amphibians; mesotocin, for fish; isotocin) has been well studied in this context. The oxytocin system functions in social recognition (Numan and Young 2016), and administration of oxytocin promotes selective partner attraction in several mammalian species (humans, Scheele et al. 2013; marmosets, Smith et al. 2010; prairie voles, Numan and Young 2016), whereas administration of oxytocin receptor antagonists constrain this behaviour (marmosets, Smith et al. 2010; prairie voles, Numan and Young 2016; zebra finches, Pedersen and Tomaszycki 2012; cichlid fish; Oldfield and Hofmann 2011). Together, these studies suggest that oxytocin signalling plays a fundamental role in pair bonding across phylogenetically diverse taxa (Fischer et al. 2019). Similarly, there is strong evidence that oxytocin and its non-mammalian homologs promote maternal and paternal care in mammals (Numan and Insel 2006), birds (Chokchaloemwong et al. 2013) and fish (O'Connell et al. 2012). There is even evidence that isotocin is a key mechanistic substrate underpinning transitions from solitary to cooperative breeding in cichlid fish (Reddon et al. 2017). Identifying and characterising the roles of other mechanisms across social structures and behaviours, including in a diverse range of taxa, represents an exciting opportunity and new perspective with which to understand the emergence and diversification of complex sociality.

The *Egernia* clade of lizards may be a useful system to start exploring these ideas. In particular, the neuropeptide arginine vasotocin (AVT) appears to be an important neurohormonal of modulator of reptile social behaviours (Zimmermann-Peruzatto et al. 2015; Lind et al. 2017; Wilczynski et al. 2017) and could be explored in this clade.

Recent work on the viviparous snake *Sistrurus millarius* has shown that blocking AVT receptors eliminates the preferences of mothers to aggregate with their offspring (Lind et al. 2017). The large within and across species variation in sociality found in the *Egernia* offers an ideal system with which to conduct manipulative experiments in combination with comparative work to explore the role of AVT (and other physiological substrates) for variation in social behaviour and organisation, especially in the context of parent-offspring associations.

Future research topic 3: Ecological and evolutionary consequences of transitions in social complexity

Over the last few decades, a large body of social evolution research (both theoretical and empirical) has been dedicated to understanding of the various ways in which complex sociality can evolve (Hamilton 1964; Trivers 1971; Axelrod and Hamilton 1981; West et al. 2002; Nowak 2006; West et al. 2002; West et al. 2007; Bourke 2011). Arguably, less effort has been paid to the ways in which sociality itself can feed back to affect both ecological and evolutionary processes.

From an ecological standpoint, it has been recognised that the highly complex sociality displayed by early humans likely played an important role in their ability to migrate from their savannah origins to colonise new, and often harsh/unfavourable ecological niches (Laland et al. 2001; Fuentes et al. 2010; Wilson 2012). The importance of eusociality for the success of social insects (accounting for probably half of the Earth's biomass of biological diversity) in colonising and occupying a range of ecosystems across the globe has also been recognised (Wilson 2012; Lucky et al. 2013). Eusociality has also been suggested to be important for explaining the broad range of habitats inhabited by snapping shrimp (Duffy and Macdonald 2010). More recently, a role of sociality in facilitating the colonisation of harsh environments in cooperative breeders has been demonstrated (Sun et al. 2014; Cornwallis et al. 2017). For example, Cornwallis et al. (2017) used a phylogenetic analysis across over 4000 bird species to show that rather than harsh environments promoting the evolution of cooperative breeding, cooperative breeding may instead facilitate the colonisation of harsh environments. Sun et al. (2014) used a series of manipulative experiments with a burying beetle (*Nicrophorus nepalensis*) to demonstrate that larger cooperative groups were able to

occupy a broader range of thermal niches and better cope with interspecific competition for resources. These studies highlight how sociality can help predict how ecological niches are colonised and may be important for predicting changing species compositions in different environments under a changing climate.

There is also a growing appreciation of the ways in which complex sociality can coevolve with other phenotypic traits. One of the most well studied of these processes is the 'social brain hypothesis' (Byrne and Whiten 1988; Dunbar 1998; Dunbar and Shultz 2007). The hypothesis posits that there are there are extensive cognitive demands involved in living in large, complex groups, which results for selection on enhanced brain size and general cognitive abilities. This hypothesis has been well tested with support from both intraspecific and comparative analyses in primates (Dunbar and Shultz 2007; Dunbar 2009, but see DeCasien et al. 2017; Powell et al. 2017 for evidence of other potentially stronger predictors of brain size), cetaceans (Fox et al. 2017) and birds (Emery et al. 2007; Shultz and Dunbar 2010; Ashton et al. 2018). Such enhanced brain size and general cognitive capacities can then coevolve with an enhanced ability to colonise and adapt to harsh environments (see above; Sol et al. 2005; Shultz and Dunbar 2010; Fox et al. 2017; Fristoe et al. 2017) and other phenotypic traits (e.g., the evolution of complex communication, Dunbar 2003). A role for sociality in driving larger brain sizes has not empirically supported in reptiles (De Meester et al. 2019) but work across and within *Egernia* lizards may uncover more nuanced consequences of sociality for cognition and enhanced size of particular brain regions (Whiting and While 2017). *Egernia* species have been demonstrated to display learning capabilities across a range of tasks (e,g, foraging and antipredation tasks) (Munch et al. 2018a, b; Riley et al. 2018; Szabo et al. 2018; Whiting et al. 2018). Further, the social setting an individual is in appears to have marked effects on their ability to perform on these cognitive tasks. For example, in *L. whitii*, associating with mothers during early postnatal periods led to improved offspring performance in an antipredation learning task (Munch et al. 2018a) and adult lizards learned a reversal task quicker when exposed to their social mate versus an unfamiliar adult conspecific (Munch et al. 2018b). Comparative work might reveal that sociality selects for enhanced cognition or uncover that other factors (such habitat complexity or predation pressure) are more important drivers of between

species differences in cognitive capabilities. Such work could then also investigate links between cognition and the development of certain regions of the brain.

The evolution of complex sociality can also have consequences for the evolution of genomes and hence have consequences for evolutionary processes more broadly. For example, most studies to date on the genetics of sociality and social behaviours have examined uni-directional relationships; that is, they have sought to identify the genetic mechanisms that underlie sociality (e.g., Walling et al. 2008; Parker et al. 2015; Bendesky et al. 2017; Kocher et al. 2018; Young et al. 2019). More recent work has highlighted that the relationship between genetics and sociality can be bi-directional; that is, sociality itself can affect genome structure and function, and thereby influence the evolution of the genome (Rubenstein et al. 2019). For example, emergent group level properties of social interactions such as caste systems can result in gene duplication and the evolution of novel genes (Samaco 2012; Chau et al. 2017). An exciting hypothesis is that social complexity, characterised by flexible behaviours, may even select for genomic properties that facilitate enhanced phenotypic plasticity such as epigenetic mechanisms or complex gene regulation (Rubenstein et al. 2019). Such genomic changes may then have broader evolutionary consequences through affecting how individuals respond to environmental change, for example by enhancing the capability of individuals to respond to changes via phenotypic and genetic accommodation (West-Eberhard 2003; Pfennig et al. 2010). These and other new novel ideas will become more amenable to testing with the growing availability of genomic resources, and further investigations in this area may help inform and advance new conceptual frameworks in evolutionary biology (e.g. the extended evolutionary synthesis; Laland et al. 2015).

Conclusion

This thesis has highlighted that examining the full range of dyadic relationships within simple family groups is crucial as researchers strive towards building a holistic and detailed understanding of the mechanisms governing the evolution of the diverse range of fraternal societies we observe in the animal kingdom. Additionally, I have outlined an integrated conceptual framework that advocates for future research to consider (a) the proximate ecological and life history mechanisms underpinning the emergence of kin groups, (b) the mechanisms – including, but not limited to kin selection – that

consolidate these kin groups, and (c) using non-traditional study systems and integrated research methodologies to help clarify the evolutionary forces that drive transitions to complex forms of social organisation. I have also discussed specific research directions that could provide novel insights and build a more detailed understanding of the evolution of complex fraternal societies and how they feedback to play a dynamic role in ecological and evolutionary processes. The potential utility of integrated experimental and comparative work across the *Egernia* clade of lizards to provide insights within each of these research directions has been highlighted. More broadly, this discussion and thesis highlight that the opportunities and prospects for new insights and synthesis in social evolution are bright.

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APPENDIX ONE

Low food availability during gestation enhances offspring post-natal growth, but reduces survival, in a viviparous lizard.

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Abstract

The environment experienced by a mother can have profound effects on the fitness of her offspring (i.e., maternal effects). Maternal effects can be adaptive when the developmental environments experienced by offspring promote phenotypes that provide fitness benefits either via matching offspring phenotype to the post-developmental environment (also known as anticipatory maternal effects) or through direct effects on offspring growth and survival. We tested these hypotheses in a viviparous lizard using a factorial experimental design in which mothers received either high or low amounts of food during gestation, and resultant offspring were raised on either high or low amounts of food post-birth. We found no effect of food availability during gestation on reproductive traits of mothers or offspring traits at birth. However, offspring from mothers who received low food during gestation exhibited a greater increase in condition in the post birth period, suggesting some form of priming of offspring by mothers to cope with an anticipated poor environment after birth. Offspring that received low food during gestation were also more likely to die, suggesting a trade-off for this accelerated growth. There were also significant effects of post-birth food availability on offspring snout-vent length (SVL) and body condition growth, with offspring with high food availability post birth doing better. However, these effects were independent of the post-natal resource environment, therefore providing no support for the presence of anticipatory maternal effects in the traditional sense.

Introduction

Maternal effects are causal effects of the mother's phenotype on offspring development (Rossiter 1996; Mousseau and Fox 1998; Räsänen and Kruuk 2007; Uller 2008; Wolf and Wade 2009). Once considered simply a source of noise when attempting to estimate heritability in quantitative genetic studies (Wade 1998), maternal effects are now recognised as having both short- and long term-term consequences for organismal development and, through this, fitness (Uller 2008; Badyaev and Uller 2009; Wolf & Wade 2009). Despite the considerable focus on maternal effects over the past decades, there is still ongoing debate regarding their adaptive significance (Mousseau and Fox 1998; Marshall and Uller 2007; Uller et al. 2013).

One way maternal effects have been suggested to be adaptive is by providing offspring with information that enables them to match the development of the appropriate phenotype to the post-birth environment (Mousseau and Dingle 1991; Mousseau and Fox 1998; Agrawal et al. 1999; Marshall and Uller 2007; Uller 2008). Such 'anticipatory maternal effects' are expected when environments are correlated across generations, allowing females to reliably transfer information about the prevailing environment to their offspring (Mousseau and Fox 1998; Uller et al. 2013). Empirical evidence for anticipatory maternal effects exists for both plants and animals. For example, wild radishes (*Raphanus raphanistrum*) exposed to non-lethal predation by caterpillars (*Pieris rapae*) increase the production of unpalatable compounds that have transgenerational effects on their offspring's ability to cope with predation (Agrawal et al. 1999). In humans, the direct negative effects of malnourishment during fetal development is partly offset by a reduction in the capacity for insulin secretion and tolerance that promotes growth and development under limited caloric intake (Hales and Barker 2001; Gluckman et al. 2008). Conversely, mismatches between the pre and post birth environment can have negative consequences for offspring fitness (Hales and Barker 2001; Metcalfe and Monaghan 2001).

An alternative (but not mutually exclusive) way that maternal effects may play out is where they have more direct effects on offspring phenotype that promote or reduce fitness across post-birth environments. For example, mothers that experience more favourable environmental conditions have been shown to produce offspring of

consistently higher fitness than mothers that experience less favourable environmental conditions (the 'silver-spoon' effect; Mousseau and Fox 1998; Kofman 2002; Hopwood et al. 2014). The opposite has also been shown, with offspring from poor maternal environments having greater fitness or size than offspring from good maternal environments, presumably as a mechanism to prepare offspring to cope with harsh conditions post birth (Taborsky 2006; Kotrschal et al. 2012). another pattern that is often observed is that offspring from mothers in a poor environment are initially a smaller size at birth, but then undergo accelerated growth to compensate (compensatory growth; reviewed in Metcalfe and Monaghan 2001). The extent to which maternal effects are manifested via anticipatory maternal effects vs. these more direct effects will determine how individuals will respond to environmental variation. It is therefore important to establish the relative importance of these different maternal effects if we are to understand their ecological and evolutionary consequences.

Resource availability is one aspect of the maternal environment that can have particularly important effects on offspring development. Indeed, the amount of resources available to offspring pre- and post-birth has been shown to affect many traits, and subsequently fitness, later in life (Kelly and Coutts 2000; Rutkowska and Cichoń 2002). Importantly, these effects can manifest themselves in multiple ways in line with the above adaptive explanations for maternal effects (e.g., Cadby et al. 2011; Itonaga et al. 2012; Wang et al. 2017). This is not surprising given that food availability often provides both the resources needed to build bodies and information about post-natal conditions (English et al. 2016). Thus, maternal food availability provides a valuable empirical context to establish the adaptive value of maternal provisioning over and above its direct resource-based effects.

Here we examine the effect of high and low food availability in both the pre- and post-birth environment on fitness-related traits of offspring in a viviparous (live-bearing) squamate, *Liopholis whitii*. To achieve this we used a fully factorial design, whereby the food available to mothers during gestation was crossed with the food available to their offspring post-birth and examined the consequences for offspring growth and survival. At the field site from where our study animals were captured (see methods below) we know that there is significant variation in climate and food availability across the breeding season and subsequent post birth period (both within and between years) and

that this may promote adaptive anticipatory plasticity. Indeed, previous research in other viviparous lizard species at the field site has found evidence of anticipatory maternal effects (Cadby et al. 2011). This system thus offers us an excellent opportunity to experimentally test the extent to which similar anticipatory were present in our study species, and compare this to evidence for other maternal effects. Combined this research will add significantly to the body of empirical research trying to understand the relative prevalence of these effects (anticipatory effects vs. silver spoon effects vs. reverse silver spoon effects vs. compensatory growth) in nature and their importance in evolutionary and ecological processes.

Methods

Study species and study animals

White's skink (*Liopholis whitii*) is a medium-sized (up to 100 mm snout vent length, SVL) viviparous skink distributed throughout a wide altitudinal range (0-1600 m) and broad habitat types in south-eastern Australia (Chapple 2003; Wilson and Swan 2017). We used *L. whitii* from a population on the east coast of Tasmania, Australia (42°57' S, 157°88' E). Individuals at this study site are found in discrete patches of open grassland in close proximity to excavated burrows or rock crevices that are used as retreat sites. *Liopholis whitii* reproduce annually, with mating occurring during the austral spring (September – October) (While et al. 2009a). Gestation spans 3-4 months and birth of offspring occurs in the austral summer (January – February).

We captured a total of 71 pregnant *L. whitii* at the end of the breeding season (early November) in 2016 using mealworm fishing and noosing techniques (outlined in detail in While et al. 2014). Pregnant females were caught after the end of the breeding season, and soon after ovulation (Cartledge and Jones 2007). This meant that females spent the majority of gestation under experimental conditions. These pregnant females were then brought to the terrestrial ecology facilities at the University of Tasmania, where they were weighed (± 1 mg), measured for length (snout-vent length (SVL), total length ± 0.5 mm) and toe clipped for permanent identification. They were then housed individually in plastic terraria (30 x 60 x 40 cm) kept under a 25 W basking light set to an 8:16 hour light/dark cycle, with overhead UV basking lights set on a 10:14 hour light/dark cycle. Each terrarium had a basking rock underneath the basking light, with a wooden shelter at the opposite end of the

terrarium. Animals were held under these conditions for a maximum of 7 days before allocation into their experimental treatments (see below).

Experimental design, food manipulations, and data collection

We carried out a fully factorial experiment whereby we manipulated food availability both during gestation and post-birth to identify their independent and interactive effects on offspring traits. The offspring traits measured were skeletal growth (measured as SVL), change in condition (measured as the scaled mass index, Peig and Green 2009; Peig and Green 2010), and mortality. The correlation between SVL and the scaled mass index was weak ($r = 0.43$, $P < 0.01$), and so we interpret these as two separate measures of offspring quality (size and condition respectively).

Our experimental design crossed high and low food availability treatments during gestation (hereafter GH and GL respectively) with high and low food availability treatments post-birth (hereafter PH and PL respectively). For the duration of both treatments the lizards were individually held in small outdoor enclosures at the University of Tasmania. These enclosures were 1 m in diameter and consisted of a Bessa block for basking, a 30 x 15 cm steel sheet metal for shelter, along with water provided *ad libitum.* All enclosures were covered with bird netting to prevent predation, although there was some mortality of offspring due to the fact that offspring are born asynchronously; this asynchronous birth establishes dominance hierarchies, leading to high amounts of aggression being directed towards the non-dominant siblings, often resulting in their death (While and Wapstra 2008; Botterill-James et al. 2017). Additionally, these enclosures were identical in terms of the vegetation within them, spaced closely together, and treatments were randomly assigned to enclosures, thereby minimising any confounding environmental effects.

We manipulated food availability by manipulating the number of mealworms each female received during their respective treatments. Females were randomly assigned to each treatment. For the gestation treatment, females in the GH treatment received five regular mealworms (size range of individual worms = 0.16 – 0.24 g) three times weekly during gestation (November until birth, which occurred from early February onwards), while females in the GL treatment received one regular mealworm three times weekly. All females in both treatments typically consumed all their food, and did so within an hour (TBJ pers. obs.) The quantity of mealworms was based on previous experiments, where adult *L. whitii* kept in the

laboratory have been shown to maintain or slightly gain mass on a diet of three regular mealworms given three times weekly (While et al. unpublished data). Examination of female body condition just after to birth suggested our treatments were successful in manipulating the amount of energy available during gestation. Specifically, females under the high food treatment during gestation had a 9.17% increase in condition, while females under the low food treatment had a -0.07% decrease in condition ($F_{(1,66)} = 74.29$, $P < 0.01$),

At the end of female gestation (mid-January), individuals were moved into indoor terrestrial ecology facilities, where they were housed individually in plastic terraria as described above. Female containers were checked at 2 h intervals daily for the birth of offspring. The average date of birth of a mother's first offspring was the same in both treatments (21/2/2017, GH range = 5/2/2017 to 16/3/2017, GL range = 3/2/2017 to 20/3/2017). For each offspring, the date of birth, weight (± 1 mg), SVL and total length (± 0.5 mm) were recorded. As all offspring retain hemipenes at birth, we were unable to determine the sex of offspring, and thus offspring sex was not considered further. Following the collection of morphometric data, offspring were toe clipped for permanent identification. The weight of mothers (± 1 mg) were recorded after birth of their last offspring. This allowed us to calculate each female's relative clutch mass (RCM; the mass of a mother's litter divided by her mass, with a mother's mass measured after the birth of all her offspring). Offspring were then released back into the outdoor enclosures with their mothers and each family was randomly assigned to a post-birth food availability treatment.

The post-birth high and low food availability treatments were identical in the magnitude of their difference to those prior to birth. However, to account for the presence of offspring along with their mother, we doubled the weight of food available in both treatments. Specifically, instead of receiving either five or one mealworms three times weekly, the enclosures received twice the equivalent weight in 'mini-mealworms' (size range of individual worms = 0.04 – 0.06 g). Mini-mealworms were substituted for regular sized mealworms as observations in the laboratory suggest offspring can choke while attempting to eat regular sized mealworms. Mothers and offspring were kept under these conditions for a six-week period after which they were returned to the laboratory where they were measured for weight and SVL, and any mortality of offspring was recorded. As with our manipulation of the pre-birth treatment, data on offspring and mother condition between the two treatments suggested that our manipulation of food availability influenced food intake (see results). Additionally, as any effects

of the post-birth environment on offspring could be mediated by mothers' behaviour in this period – i.e., mothers who received low food during gestation could consume more food in the post-birth environment, therefore depriving offspring of resources. To check this, we analysed whether the gestation treatment affected mother's change in condition from birth until the end of the experiment, but found no effect. Further, there was no effect of a mother's postnatal change in weight on the change in SVL or condition of offspring. At completion of the experiment, all mothers and their offspring were released back to their mother's initial site of capture at the field site.

Across the four treatments our sample sizes per treatment group for the experiment were GH-PH = 19 females, GH-PL = 18 females, GL-PH = 16 females and GL-PL = 18 females. Of these 71 females, 59 produced offspring (29 in the GH treatment and 30 in the GL treatment). In total, 117 offspring were produced, although two of these were unhealthy and died early in the laboratory before any measurements were taken. The total number of offspring included in the experiment was therefore 115 (per treatment group: GH-PH = 28, GH-PL = 31, GL-PH = 29 and GL-PL = 27).

Data analyses

Data were analysed using a combination of ANOVAs, Generalized Linear Models (GLMs), Linear Mixed Models (LMMs) and Generalized Linear Mixed Models (GLMMs) fit by maximum likelihood. These were run in R version 3.4.1 (R development core team 2017). For the mixed models, Laplace approximation was used to estimate model parameters, as it is a more accurate technique than the simpler and widely used pseudo quasi-likelihood estimation method (Bolker et al. 2009). All fixed effects were tested with *Z*, Wald's $\chi 2$ or type III *F*- tests (with Kenward-Rogers approximation used for *F*-tests in LMMs).

We first examined the effect of the gestation treatment on female reproductive traits and offspring traits at birth. For female reproductive traits, we ran three models, all with the gestation treatment as the sole predictor variable: 1) a GLM with a binomial family and a logit link, and the probability of a female producing a litter (yes/no) as the response variable, 2) an ANOVA with female RCM as the response variable, and 3) an ANOVA with offspring date of birth as the response variable. For offspring traits at birth, we analysed whether there was any effect of the gestation treatment on offspring SVL or condition at birth, using LMMs with gestation treatment as a fixed effect, birth date as a covariate, and maternal identity as a random effect.

Both offspring mass and SVL displayed right skew and so were log transformed prior to analyses.

Second, we ran three models to examine the effect of the food availability treatments on offspring traits at the end of the six week post-birth treatment: 1) a LMM with change in offspring SVL from birth until the end of the experiment as the response variable, 2) a LMM with change in offspring condition from birth until the end of the experiment as the response variable, and 3) a GLMM with offspring mortality as the response variable (dead or alive for each offspring after six weeks). All models included gestation treatment, post-birth treatment and the interaction between pre- and post-birth treatment as fixed effects, and maternal identity as a random effect to account for non- independence arising from litters containing multiple offspring. For the models including offspring SVL and condition we included offspring SVL and condition at birth as covariates respectively. We also included offspring date of birth, as this has previously been shown to significantly influence early offspring growth (While et al. 2009b). Further, we included whether any of an offspring's siblings died, to control for the potential effect of this on reducing food competition and increasing growth. For the mortality model we included initial offspring SVL to exclude any potential differences of growth of offspring between gestation treatments being due to effects of viability selection (results were similar when condition at birth was used as a covariate instead of SVL). We also included relative clutch mass as a covariate in these models, but this did not affect our results, so we report our results with this excluded for simplicity. Both offspring mass and SVL displayed right skew and so were log transformed prior to analyses.

All results are reported as means with standard errors, and we report results for models containing all main effects and significant interactions following backward elimination of non-significant interaction terms. All models were checked for violation of assumptions (visual inspection of residuals plots for heteroscedasticy and non-normality of errors) with no violations being found. For our survival model, we checked goodness of fit by computing McFadden's R^2, which was 0.14, indicating an adequate fit.

Results

We found no effect of food availability during gestation on any component of female reproductive output, with no effect on the probability of producing a litter (GH: 82%, GL: 88%), RCM (GH: 0.18 ± 0.01, GL. 0.19 ± 0.01), or date of birth (GH: 23/2/2017, GL: 21/2/2017) (Table

1), despite strong effects on maternal condition (see above). Similarly, we found no effect of food availability during gestation on offspring condition at birth (GH: 36.48 ± 0.49 mg/mm, GL: 36.53 ± 0.49 mg/mm) or size at birth (GH: 35.78 ± 0.16 mm, GL: 36.34 ± 0.20 mm) (Table 1). In contrast, we found a significant effect of food availability during gestation and in the first six weeks following birth on offspring change in condition, but not SVL. Specifically, we found that offspring whose mothers experienced low food availability during gestation grew better from birth until the end of the experiment than offspring whose mothers experienced high food availability during gestation, independent of their post-birth environment (GH: 0.04 ± 0.01 change in scaled mass index GL: 0.12 ± 0.03 change in scaled mass index) (Figure 1, Table 2). We also found that offspring who had access to high food availability in the six weeks following birth had significantly greater SVL and condition growth than offspring who had access to low food availability (PH: 3.29 ± 0.34 mm change in SVL, PL: 1.94 ± 0.26 mm change in SVL, PH: 0.11 ± 0.01 change in scaled mass index, PL: 0.04 ± 0.02 change in scaled mass index) (Figures 1 and 2, Table 2). These effects of the post-birth environment were independent of any effects of the gestation treatment on these traits – that is, we found no interactive effects of pre- and post-birth food availability on either offspring SVL or condition (Table 2). Additionally, there were no significant effects of litter level mortality on offspring growth, i.e., an individual offspring's growth was independent of the mortality of its siblings. Therefore, these food availability effects on offspring growth can be interpreted as true effects rather than simply an artefact of differential offspring mortality between treatments (see below).

Overall, offspring mortality was 34 %, which is similar to rates in natural populations (While et al. 2009a) and in previous captive population experiments (Botterill-James et al. 2016, Halliwell et al. 2017). We found a significantly higher mortality of offspring from the low pre-birth food availability treatment compared to the high pre-birth food availability treatment, but not between the post birth treatments or any interactive effects between pre and post-birth treatments (GH-PH = 22%, GH-PL = 28%, GL-PH = 41% and GL-PL = 48%) (Table 2). We also found that offspring that were smaller at birth were more likely to die (average SVL for survivors: 36.17 ± 0.14 mm, average SVL for offspring that died: 35.60 ± 0.22 mm), but there was no interactive effect with the gestation treatment (Table 2).

Table 1. Parameter estimates with standard errors, test statistics, and P-values for models examining treatment effects on female reproductive traits and offspring snout-vent-length (SVL) and condition at birth. Significant effects are bolded.

	Reproduced or not			**Relative Clutch Mass**			**Birth date**			**Offspring birth SVL**			**Offspring birth condition**		
	estimate	std. err	P	estimate	std. err	P	estimate	std. err	P	estimate	std. err	P	estimate	std. err	P
Intercept	**$Z_{(1)}$ = 3.225**			**$F_{1(,54)}$ = 278.54**			**$F_{1(,55)}$ = 153.10**			**$F_{1(,53.94)}$ = 92195.76**			**$F_{1(,51.57)}$ = 44.33**		
	1.29	**0.40**	**< 0.01**	**0.18**	**0.01**	**< 0. 01**	**22.5**	**1.82**	**< 0.01**	**1.56**	**0.01**	**< 0.01**	**0.15**	**0.02**	**< 0.01**
Gest treat	$\chi 2_{(1)}$ = 1.19			$F_{1(,54)}$ = 0.14			$F_{1(,55)}$ = 0.17			$F_{(1,51.97)}$ = 1.60			$F_{(1,51.09)}$ = 0.81		
(low)	0.73	0.67	0.27	< 0.01	0.02	0.71	-1.05	2.55	0.68	< 0.01	< 0.01	0.21	-0.01	0.02	0.37
Birth date										**$F_{(1,52.46)}$ = 4.67**			$F_{(1,49.30)}$ = 2.73		
										< -.01	**< 0.01**	**0.04**	< -.01	< 0.01	0.10

Figure 1: Effects of gestation and post-birth treatments on offspring change in condition (scaled mass index). Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. n (from left to right) = 21, 19, 16, 12.

Table 2 Parameter estimates with standard errors, P-values and test statistics for models examining treatment effects on offspring growth and survival. Significant effects are bolded. Main effects are from models with interactions excluded

	Offspring SVL change			**Offspring condition change**			**Offspring mortality**		
	estimate	std. err	P	estimate	std. err	P	estimate	std. err	P
Intercept	**$F_{(1,60.37)} = 7.33$**			**$F_{(1,39.16)} = 47.76$**			**$Z_{(1)} = 2.18$**		
	0.71	**0.25**	**< 0.01**	**0.26**	**0.04**	**< 0.01**	**57.29**	**26.35**	**0.03**
Gestation treatment	$F_{(1,35.46)} = 1.33$			**$F_{(1,31.59)} = 6.24$**			**$\chi2_{(1)} = 8.60$**		
(low)	0.01	0.01	0.29	**0.05**	**0.02**	**0.02**	**1.44**	**0.50**	**< 0.01**
Post-birth	**$F_{(1,32.40)} = 7.54$**			**$F_{(1,32.15)} = 19.93$**			$\chi2_{(1)} = 0.05$		
(low)	**-0.01**	**<0.01**	**<0.01**	**-0.09**	**0.02**	**< 0.01**	0.10	0.45	0.82
Interaction	$F_{(1,33.44)} = 0.15$			$F_{(1,33.56)} = 0.06$			$\chi2_{(1)} < 0.04$		
	< 0.01	0.01	0.70	0.01	0.04	0.81	0.20	0.98	0.84
Initial SVL (SVL	**$F_{(1,60.93)} = 6.25$**			**$F_{(1,60.69)} = 38.24$**			**$\chi2_{(1)} = 5.03$**		
condition	**-0.42**	**0.16**	**0.02**	**< -.77**	**0.12**	**<0.01**	**-37.94**	**16.92**	**0.02**
Birth date	**$F_{(1,33.97)} = 11.77$**			**$F_{(1,34.18)} = 9.22$**			$\chi2_{(1)} = 0.09$		
	< -.01	**< 0.01**	**< 0.01**	**< -.01**	**< 0.01**	**< 0.01**	< 0.01	0.02	0.77
Gestation treatment							$\chi2_{(1)} = 0.94$		
Initial SVL							24.88	33.46	0.46
Litter level	$F_{(1,40.56)} = 0.12$			$F_{(1,40.08)} = 0.41$					
	< 0.01	< 0.01	0.73	-0.01	0.02	0.53			

Figure 2: Effects of gestation and post-birth treatments on offspring change in SVL (mm). Numbers in circles represent sample sizes. Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. n (from left to right) = 21, 19, 16, 12.

Discussion

Here we found no effect of food availability during gestation on reproductive traits of mothers or on offspring traits at birth. However, we did find a strong effect of food availability during gestation on both offspring growth and survival. Specifically, offspring from mothers that received low food during gestation increased their condition more during the first 6 weeks of life than offspring from mothers that received high food during gestation, but those offspring were also more likely to die. We also found that offspring grew better when given high food post birth, but there were no interactive effects of the pre and post treatments. Combined, these results do not support the idea of anticipatory maternal effects in the traditional sense; rather, they suggest that mothers that receive a low amount of food during gestation may anticipate a poor environment post-birth and prime their offspring for better growth, but that this comes at a trade-off for survival. We discuss these results in the context of a growing body of literature on the adaptive significance of maternal effects.

We found no evidence that the resource availability mothers experienced during gestation mediated key offspring traits at birth. There are several potential explanations for this lack of effects. First, females may be buffering offspring from the poor resource conditions during gestation such that offspring fitness is not compromised (see Groothuis & Taborsky 2015). Indeed, there is some precedence for maternal buffering of

offspring in viviparous reptiles. This has mainly been studied in the context of sub-optimal thermal environments (Gvozdik 2002; Doody et al. 2006; Telemeco et al. 2009), including in a viviparous species found at our site (*Niveoscincus ocellatus*, Uller et al. 2011), but could also occur if females alter their resource allocation during gestation to offspring based on the conditions they experience. Alternatively, the lack of effects of the pre-natal resource environment on offspring traits could be the outcome of aspects of this species' biology that mean females have limited capacity to modify the amount of raw resources to offspring. *Liopholis* species posess relatively a simple placenta (Chapple 2003), which means that the majority of resources offspring rely on for early development is contained within the yolk, potentially limiting the capacity for large transfer of resources during gestation compared to similar species with more complex placentas (e.g., Itonaga et al. 2012).

While we found no evidence that conditions during gestation mediated offspring traits at birth, we did find strong evidence that the resources available to mothers during gestation influenced offspring growth post-birth. Offspring from females held in resource poor environments during gestation grew better in terms of their body condition than offspring from females held in resource rich environments. These results match findings in other viviparous lizards (Caley and Schwarzkopf 2004; Itonaga et al. 2012; Wang et al. 2017; but see Shine and Downes 1999), as well as many other taxa (Metcalfe and Monaghan 2001). Combined, these results suggest that while resource transfer during gestation is not enough to alter offspring traits at birth, it can mediate a range of post-birth processes. For example, mothers may be anticipating a poor environment for their offspring when experienceing low food during gestation and priming their offspring for increased growth post birth (e.g., Taborsky 2006). This could be achieved in several ways, such as an increase in growth hormone levels/growth hormone receptors (e.g., Segers et al. 2012) or earlier and/or greater development of organs involved in digestion (e.g., Kotrschal et al. 2014). More work is required to fully understand the mechanisms that link the resource conditions experienced by females during gestation and the processes that lead to enhanced growth of offspring in the post-natal environment.

Regardless of the mechanism(s), such effects may lead to trade-offs whereby the enhanced ability to capitalise on limited resources has functional consequences elsewhere. For instance, in the multi-ocellated racerunner (*Eremias multiocellata*), offspring from mothers with

restricted food during gestation exhibited greater growth but also had lower locomotor performance, suggesting costs to this increased growth response (Wang et al. 2017). In line with these results, we also observed a trade-off in the low food gestation treatment. Specifically, while offspring exhibited enhanced growth they also exhibited increased mortality. Further work is required to understand the mechanisms underlying this trade-off in our system. One potential mechanism is that pre-birth conditions influenced the level of competition in the brood post-birth (Muller et al. 2012). This could occur through either direct allocation of resources that mediate levels of aggression (e.g., testosterone; Muller et al. 2012) or via increases in birth spread (While and Wapstra 2008). Both may result in higher competition for resources in the post-birth environment, leading to higher growth, but would also cause higher mortality (as sibling aggression is high and a key source of offspring mortality, While and Wapstra 2008; Botterill-James et al. 2017). Similarly, mothers may be stressed by low food during gestation, causing them to attack offspring. However, we did not observe higher sibling aggression in the low food gestation treatment (Botterill-James et al. in prep) nor did we observe mothers attacking offspring (Botterill-James et al. in prep; see Botterill-James et al. 2017 for evidence of lack of maternal aggression in this system).

There are several alternative explanations for the observed increased in condition in the low food gestation treatment, but we believe these can safely be ruled out. One alternative explanation is that offspring from mothers in the low food gestation treatment may have been better growers simply because selective mortality removed the smaller and weaker individuals. Indeed, we found that individuals that were smaller at birth were more likely to die, and mortality was higher in the low food gestation treatment. However, three lines of evidence suggest that this is an unlikely explanation for our results. First, offspring were the same size and condition at birth between the low and high food gestation treatments. Second, there was no evidence that the effect of body size on offspring survival was greater in low food gestation treatment compared to the high food gestation treatment. Finally, the best growing individuals in the low food gestation treatment had greater growth than any of the best growing individuals in the high food gestation treatment. Combined, these results support our interpretation that mothers experiencing low food during gestation prime their offspring to grow better after birth but that this then trades off with another aspect/s of offspring quality,

causing higher mortality. Another alternative explanation for the observed increased in condition in the low food gestation treatment is that the increased mortality in this treatment reduced food competition, thereby allowing for increased growth of those surviving offspring. However, we can rule this out, as in our statistical models of offspring growth, there was no significant effect of litter level mortality on offspring growth, i.e., an individual offspring's growth was independent of the mortality of its siblings. (see table 2). A final alternative explanation for the observed increase in condition of offspring from the low food gestation treatment is that offspring could have grown more if mothers were physically weakend by the low availability during gestation, and thereby not have been able to compete well with offspring for access to food. However, this is highly unlikely; mothers are an order of magnitude larger than their offspring at birth, and we have never observed adults being outcompeted or subjected to aggression by offspring either in the field (While pers obs) or under captive conditions (Botterill-James et al. 2017). We also did not observe any differences in health or behaviour of mothers between the two gestation treatments. Vice versa, mothers who received low food during gestation could consume more food in the post-birth environment, therefore depriving offspring of resources. This pattern is counter to our results however, and we found no effect of the gestation treatment on a mother's change in condition from post birth to the end of the experiment, or any effect of a mother's postnatal condition change on offspring traits.

In contrast to the above effects, we found no evidence for the presence of an interactive effect of the pre- and post-birth resource environment on either offspring growth or condition. This suggests that anticipatory maternal effects may be unlikely to play a role in mediating offspring fitness in this system. These results are consistent with several recent studies on both viviparous (Wang et al. 2017) and oviparous (Warner et al. 2015) lizards which have also failed to find evidence for anticipatory maternal effects in the context of resource availability manipulation (but see Cadby et al. 2011). There are several potential explanations for this lack of support. First, the maternal environment may not predict the environment that offspring experience post birth. Second, the environment may not be sufficiently variable to render adaptive plasticity beneficial (Burgess and Marshall 2014). Despite these being explicit pre-requisites of anticipatory maternal effects (Uller et al. 2013), they are very rarely measured (Taborsky 2017), which may explain why, in many instances, the responses do not exhibit the trade-off

across environments that is expected for adaptive plasticity (e.g., Uller et al. 2013; Engqvist and Reinholdt 2016). While previous work has shown the presence of anticipatory maternal effects in other viviparous species at our study site in the context of food availability (Cadby et al. 2011), we do not have explicit information on the extent to which food availability during gestation matches resource availability in the post-birth environment. Given that key climatic conditions at our site can vary considerably between the pre- and post-birth environment (Wapstra et al. 1999), there may limited predictability in conditions between these time points. Additionally, there may be components of *Liopholis whitii's* sociobiology that have an impact on the predictability of resources for individual offspring, influencing selection on anticipatory maternal effects. *Liopholis whitii* exhibit simple forms of post-birth parent-offspring associations, with considerable variation within a litter with regards to the extent of these associations. Specifically, one offspring typically associates with its parents (sometimes for up to several years, Chapple & Keogh 2006; While et al. 2009; Botterill-James et al. 2016), whereas its siblings will often disperse. Thus, these differences between siblings in the post-birth social environment may modify the extent to which individuals are exposed to predictable or non-predictable environmental effects, thereby mediating the strength of selection on anticipatory maternal effects.

In summary, we found that mothers that experienced poor conditions during gestation primed their offspring for better growth post birth. Instead of trading this off against relative clutch mass or offspring number, this came at a cost to offspring survival, with higher mortality of offspring from mothers receiving low food during gestation. Teasing out the mechanisms responsible for these effects and the evolutionary and ecological consequences of such effects provide exciting avenues for future research.

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Data accessibility

Data available from Dryad Digital Repository (Botterill-James et al. 2018).

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APPENDIX TWO

Maternal effects impact decision-making in a viviparous lizard

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Abstract

Stressful conditions experienced during early development can have deleterious effects on offspring morphology, physiology and behaviour. However, few studies have examined how developmental stress influences an individual's cognitive phenotype. Using a viviparous lizard, we show that the availability of food resources to a mother during gestation influences a key component of her offspring's cognitive phenotype; their decision-making. Offspring from females who experienced low resource availability during gestation did better in an anti-predatory task that relied on spatial associations to guide their decisions, whereas offspring from females who experienced high resource availability during gestation did better in a foraging task that relied on colour associations to inform their decisions. This shows that the prenatal environment can influence decision-making in animals, a cognitive trait with functional implications later in life.

Introduction

Cognitive processes allow animals to perceive, consolidate and act on information acquired during development – playing a fundamental role in an organism's ability to address environmental challenges [1, 2]. Despite this, our understanding of what drives individual variation in cognitive traits is limited [3]. As a consequence, we are ill-equipped to understand the role intra-individual variation in cognition plays in mediating evolutionary and ecological processes.

It has long been suggested that stressors experienced in early life impact offspring phenotypic traits, often with profound and long-term effects [4, 5]. Indeed, the early developmental environment affects a range of morphological, behavioural and physiological traits [6]. Early life stressors have also been suggested to shape an individual's cognitive phenotype due to differential or impaired allocation of resources to brain formation [7]. Despite this, few empirical studies have examined early environmental effects on the development of cognitive traits themselves (but see [1]).

Here we use a viviparous lizard to examine how food availability during two periods early in development, pre- and post-birth, affects offspring decision-making and learning. To achieve this, we subjected offspring raised on low or high resource availability to two cognitive tasks, a 'foraging task' that relied on colour associations and an 'anti-predatory task' that relied on spatial associations.

Methods and materials

(a) Animal collection and experimental design

We collected 70 pregnant female *Liopholis whitii* from Orford, Tasmania, at the start of gestation. Females were transported to the University of Tasmania, measured for morphometric traits [weight (± 1 mg) and snout-vent-length (SVL; ± 0.5 mm)] and housed in outdoor enclosures (1 m diameter). Females were then randomly allocated to one of two pre-birth resource treatments. Females in the low resource treatment were provided one mealworm (*Tenebrio molitor*) three times weekly, whereas females in the high pre-birth treatment received five mealworms three times weekly. These treatments resulted in significant differences in female body condition at the end of gestation (ANCOVA: $F_{1,65} = 68.60$, p-value < 0.001).

At the end of gestation (mid-January) females were moved to indoor terrestrial ecology facilities to give birth. At birth, we recorded offspring morphometric traits. We were unable to identify offspring sex as juvenile female *Liopholis* retain their hemipenes until sexual maturity. Females and their offspring were then randomly assigned to a post-birth treatment that was either the same or the opposite to the one the female experienced during gestation. In the post-birth treatment, we doubled the number of mealworms provided to account for the presence of both the female and her offspring. Females and their offspring were released into the small outdoor enclosures and kept under the post-birth resource treatments for a six-week period. At the completion of this six-week period, offspring were returned to the laboratory where they were remeasured for morphometric traits and housed individually. We then assayed all offspring for two cognitive tasks.

(b) Cognitive tasks

We subjected offspring to two cognitive tasks to test for differences in their ability to solve these tasks and to test whether differences were dependent on the context of the task and the cues available to inform decisions. The order of assays was randomized across all offspring. For both tasks, offspring were assayed twice daily (morning and afternoon) over 10 consecutive days (n = 20 trials per cognitive task). We scored the offspring's first choice (correct vs incorrect). All assays were scored blind to the offspring's treatment.

Foraging task: Offspring had to learn to associate a food reward with a specific coloured block [8]. For each trial, we placed a food dish on each of two elevated blocks [7 (L) x 7 (W) x 4 (H) cm], one blue and one white in front of the offspring such that the food could not be seen. One block allowed access to a mealworm, while access to the mealworm in the other block was prevented by a mesh screen inside the dish. We gave the offspring a maximum of 1 h to attempt the task. We considered the offspring to have made a choice if it placed both its forelimbs on the top edge of the block. The colour and position (right or left) of the correct block was randomized and counter-balanced across treatment groups to account for colour or spatial bias between offspring.

Anti-predatory task: We set up a biologically relevant anti-predatory paradigm, which has been used with success in previous studies [9]. In this task, offspring had to learn the

location of a 'safe' refuge when given the option of two refuges. Specifically, we simulated predatory attacks using a standardized protocol (tapping the offspring from behind on the pelvic girdle using our index finger) allowing the offspring to choose its flight direction until it entered the 'safe' refuge. If it entered the 'unsafe' refuge, we lifted the refuge and resumed chasing (the refuge was immediately replaced in its original position). We gave the offspring a maximum of 10 min to complete the task. We considered offspring to have made a choice when it ran inside a refuge. We kept the location of both refuges constant throughout the trials to determine whether offspring use spatial cues to solve the task but randomized the location of the 'safe' refuge between offspring within each resource treatment.

Statistical analysis

We analysed differences in decision-making (overall probability of choosing correct) and learning rates (increase in probability of choosing correct across trials) using generalized linear mixed models in the lme4 package [10] in R v 3.0.3 [11]. Within our models we included pre- and post-birth treatment and their interaction, z-transformed trial and z-transformed SVL at 6 weeks as fixed factors and individual and maternal identity as random effects. We also included an interaction between treatment and trial to test for differences in learning rates and considered an increase in the probability of making correct choices across trials as evidence for learning. The inclusion of a random slope (trial) in our models led to poor model convergence and was dropped from subsequent analyses. Model reduction was performed following backward elimination of non-significant interactions ($p > 0.05$) and resulted in main effects models. We tested for lateralization bias and none were found. See the electronic supplementary material (ESM) for full details on methodology and statistical analyses.

Results

We found a significant effect of pre-birth resource treatment on the probability of choosing correctly in both tasks, however, the direction of the effect was opposite for the two tasks (Table 2.1). Offspring from the low pre-birth treatment had a higher than expected probability of choosing correct in the anti-predatory task and differed significantly from offspring developing under high pre-birth resources. This pattern was reversed in the foraging task, where offspring from the high pre-birth treatment had a

higher than expected probability of choosing correct – differing significantly from offspring developing under low resources (Figure 2.1). We found no evidence that the probability of offspring choosing correctly increased across trials, indicating that the cognitive process affected was decision-making rather than learning the tasks *per se*. We found some evidence that this lack of learning was a result of over-training or changed motivation across trials (i.e., modelling a non-linear – quadratic– trial effect) in the foraging task, but found no evidence for this in the spatial task (see ESM). We found no effect of the post-birth resource treatment on the probability of choosing correctly in either task (Table 2.1).

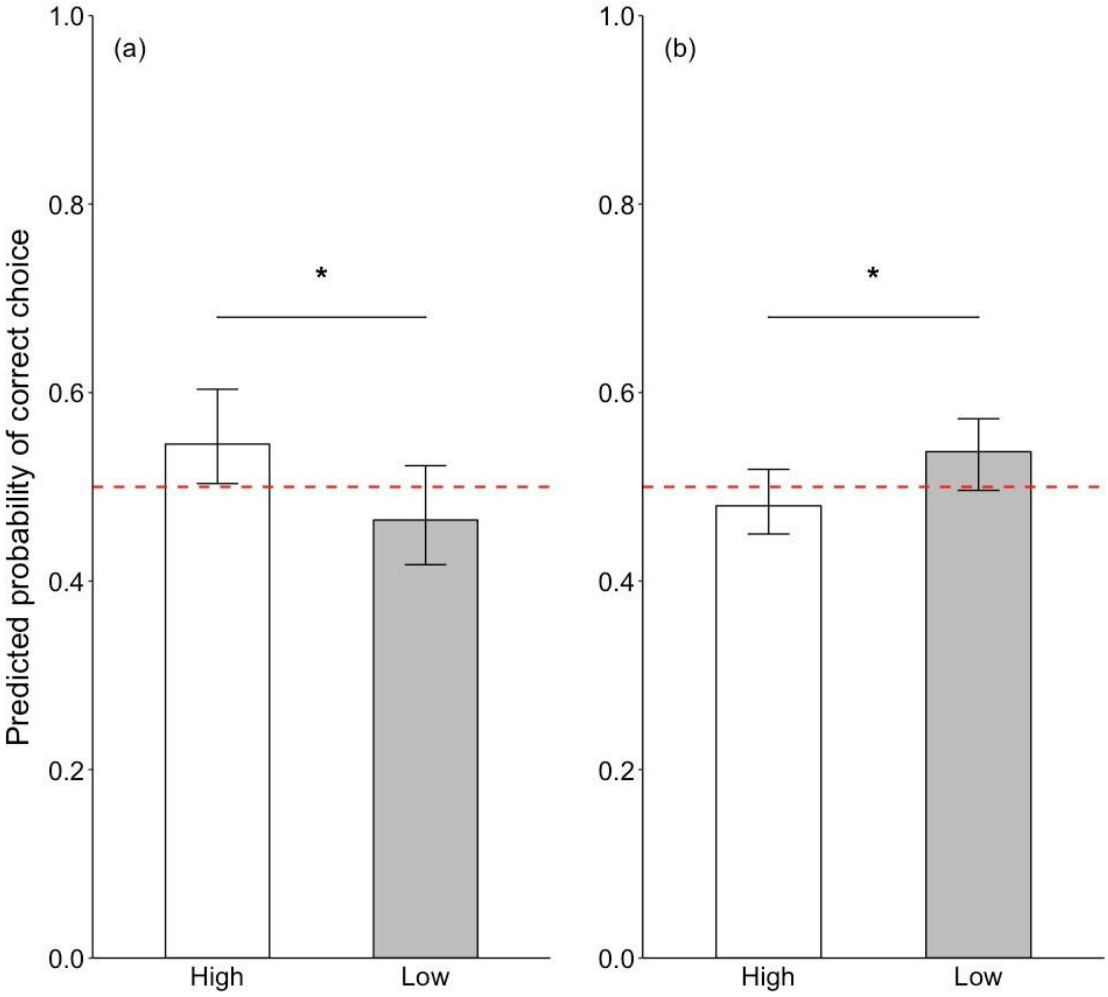

Figure 2.1 Mean (± 95% CI) predicted probability of choosing correct in (a) a foraging and (b) an anti-predatory task for offspring exposed to high or low pre-birth resource availability. The dashed line represents 50/50 chance. * Differences significant at $p < 0.05$.

Table 2.1 Parameter estimates and 95% CI of GLMMs examining the relationship between pre- and post-birth resource availability on offspring's probability of choosing correct in a foraging and anti-predatory task.

	Foraging task				**Anti-predatory task**			
	estimate (β)	lower CI	upper CI	*p*-value	estimate (β)	lower CI	upper CI	*p*-value
Intercept	0.25	-0.03	0.54	0.07	-0.05	-0.22	0.12	0.57
Scaled SVL	**0.21**	**0.05**	**0.38**	**0.01**	-0.10	-0.21	0.01	0.08
Scaled trial number	0.07	-0.04	0.19	0.22	0.02	-0.09	0.12	0.73
Treatment pre-birth LOW	**-0.41**	**-0.77**	**-0.05**	**0.02**	**0.25**	**0.02**	**0.47**	**0.03**
Treatment post-birth LOW	0.007	-0.33	0.36	0.97	-0.07	-0.28	0.14	0.52
Scaled trial × pre-birth	0.05	-0.18	0.28	0.67	0.11	-0.10	0.32	0.30
Scaled trial × post-birth	-0.02	-0.25	0.21	0.85	0.002	-0.21	0.21	0.30
Pre- × post-birth	0.03	-0.67	0.72	0.92	0.001	-0.57	0.29	0.99

Statistically significant results are in bold. Main effects are presented from a model without interactions.

Discussion

Our results suggest that the resource environment a mother experiences during gestation influences offspring decision-making. Offspring exposed to low prenatal resource availability did better in the anti-predatory task with spatial associations to guide decisions, while offspring that developed under high prenatal resource availability did better in the foraging task with colour associations as guidance. These results were independent of any clear evidence of learning to solve these particular tasks, suggesting that the prenatal environment influenced decision-making patterns rather than task-specific learning.

The absence of learning in these tasks is surprising given that previous studies on adult *L. whitii* have been shown to them capable of learning both cognitive tasks (KL Munch unpublished data). While changed motivation or over-training may explain a lack of learning in the foraging task, it remains unclear why no learning was detected in the anti-predatory task. There are a number of potential explanations for this, including age-specific constraints [12] or an inability to cope with stress, which is known to affect learning [13]. Alternatively, learning may have occurred but in more nuanced ways that we failed to pick up with our experimental design. Indeed, the non-linear relationship between probability of correct choice and trial number in the foraging tasks suggests more subtle effects on learning may be occurring. More data on how developmental conditions influence learning *per se* is required to tease apart these alternative explanations.

Irrespective of the lack of task-specific learning, the broad effect of the prenatal environment on the development of cognitive traits in *L. whitii* is in agreement with effects in other taxa. Indeed, studies have shown that a range of prenatal conditions (e.g., malnutrition, hormone exposure) can have substantial impacts on the development of key traits associated with an individual's cognitive ability (e.g., learning and memory, see [1] for review). For example, geckos incubated in hot temperatures had poorer spatial learning ability, than geckos incubated in colder temperatures [14]. However, despite a consistent overall effect of prenatal conditions impacting development of decision-making, the relative direction of these effects differed depending on the cognitive task, with offspring experiencing poor resource conditions during gestation

making more correct decisions in the anti-predatory task but worse in the foraging task. One explanation for these results is that natural selection has shaped the developmental trajectory of decision-making in relation to the likely resource environments offspring find themselves in post-birth (i.e., a context-dependent anticipatory maternal effect [15]). More work targeting whether the subtle changes we see in decision-making between treatments translate into fitness benefits under different postnatal conditions is required to tease apart these explanations. Alternatively, these effects may be a non-adaptive consequence of differential resource allocation to different areas of the brain. In reptiles, the spatial domain is linked to the medial cortex while visual information (such as colour) is processed in the dorsal cortex [16]. Therefore, any trade-off between resource allocation to these areas of the brain for other functional reasons may feedback to influence decision-making. The integration of functional outcomes of cognitive traits with a fundamental understanding of the neural biology underlying those traits is lacking for the majority of non-model systems but will provide a fruitful avenue for future research.

We found no evidence for effects of postnatal environment on the probability of choosing correctly in either cognitive task. As all offspring were held with their mothers in the postnatal environment we cannot rule out potential carry over effects of the prenatal environment on maternal behaviour that may in turn have masked any effects of the postnatal environment. However, the strong effects of the postnatal environment on other aspects of offspring development (e.g., improved offspring SVL growth; see ESM) suggests that the lack of effects is likely to be real.

In summary, we found that decision-making is affected by prenatal resource conditions. While our data do not provide insight into the mechanism(s) responsible or the fitness consequences, they suggest that early life stressors may evoke a trade-off between allocating resources to developing different cognitive domains, enhancing one at the expense of another.

Ethics

Research approved by the University of Tasmania Animal Ethics Committee (A0016084)

Data accessibility

The dataset is available at the Dryad digital repository: https://doi.org/10.5061/dryad.01vc6.

Author's contributions

KLM, DWAN, TBJ, EW and GMW conceived the study. Data were collected by KLM, ISK, BH, and TBJ. KLM, DWAN and GW performed the analysis. All authors wrote the manuscript, approved the version to be published and agreed to be accountable for all aspects of the research.

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Electronic supplementary material

Extended methods and materials

Animal capture and husbandry

We captured 70 pregnant female *L. whitii* from wild populations on the east coast of Tasmania, Australia (42◦57′S, 147◦88′E) at the start of gestation (early November) in 2016. Upon capture, females were returned to the University of Tasmania where they were measured for snout-to-vent length (SVL) and body mass (± 1 mg). Females were then housed individually in opaque tubs [57 (L) x 38 (W) x 32 (H) cm], provided with cat litter as a substrate (~ 5 cm thick) and a rock as a basking site and a refuge. Basking lights (25W) and overhead UV lighting set to 0800-1800 h night/day cycle provided thermoregulatory opportunities. Following assignment of resource treatment (see below), pregnant females were moved to small outdoor enclosures (1 m diameter) at the animal compound at the University of Tasmania. Enclosures were covered with bird netting to prevent predation, and each enclosure was supplied with a brick block for basking, a steel sheet (30 x 15 cm) for shelter, and water provided *ad libitum.*

Resource manipulation

Resource availability was manipulated both during gestation (i.e., 'pre-birth' resource availability) and for the six weeks following the birth of all offspring (i.e., 'post-birth' resource availability) using a fully factorial design. In the pre-birth treatment, females in the low resource availability treatment were given access to one mealworm at three-time points during the week. This was based on previous experiments which have shown that adult *L. whitii* maintain or slightly gain mass on a diet of three regular mealworms (*Tenebrio molitor*) given three times weekly in the laboratory (While *et al.* unpublished data). Thus, this set the lower level for resource availability. In contrast, females in the high pre-birth treatment received five regular sized mealworms three times weekly. We controlled feeding by always placing mealworms on top of the brick block in a feeding dish in order to ensure that they were all eaten. Mealworms were supplied by Pisces Enterprises. To confirm our resource treatment had worked, we examined differences in female body condition at the end of gestation between females held under the two resource treatments. We found that females from the high resource availability treatment were in significantly better condition [i.e., gained more body mass (g)] than females

from the low resource availability at the end of the experiment (ANCOVA: $F_{1,65} = 68.60$, *p*-value < 0.001). We included SVL as a covariate to account for any differences in body size.

Females were held under the above conditions until the end of gestation (mid-January). At the end of gestation, females were recaptured from the small enclosures, moved back to the laboratory and housed as described above. We checked females daily at 2 h intervals for offspring. For each offspring, we recorded the date of birth, weight (± 1 mg) and SVL (± 0.5 mm). One important caveat with our experimental design is that differences in resource availability may influence offspring development indirectly, through any effects on the behaviour of females, specifically thermoregulatory effects. Importantly, previous work showed that thermal conditions during development can have significant effects on cognitive development [1, 2]. While we did not record differences in female thermoregulatory behaviour during this time, we can test for this by examining differences between treatments in offspring birth date. Embryonic development, and hence gestation length, is temperature dependent in lizards [3]. Given all females were captured after ovulation (thus we expect no differences between treatments in time of ovulation) any differences between treatment in birth date should be a good indicator of the differences in the temperature experienced during embryonic development [4]. Nonetheless, we found no differences between females from the two treatments in offspring birth date (ANOVA, $F_{1,55} = 0.17$, p-value = 0.68). This suggests that any differences in offspring phenotype post-birth are likely be driven by resource availability *per se* as opposed to an effects of resource availability female thermoregulatory behaviour. In addition, we found no difference between treatments in the size of the clutch female produced (ANCOVA with female SVL as a covariate: $F_{1,4.02} = 14.44$, *p*-value = 0.21), suggesting that differences in the offspring's pre- and post-birth competitive environment were unlikely to influence the results.

Following birth, each mother and her offspring were randomly assigned to either the same or opposite resource treatment to which the mother had experienced during gestation. Mothers and their offspring were then released back into the outdoor enclosures, and the post-birth food availability manipulation began. We doubled the resource availability treatment in the post-birth treatment to account for the presence of offspring and their mother (as opposed to just the mother). Furthermore, as juvenile *L. whitii* may choke on full sized mealworms, mothers and offspring received the equivalent weight in 'mini-mealworms' as opposed to regular sized mealworms. Offspring and their mothers were then maintained on the post-birth treatment for

6-weeks. Once a week, each offspring was measured for body mass, body size (SVL) and mortality.

Following completion of the resource availability treatment, offspring were returned to the laboratory to be re-measured and undergo cognitive tasks. During this period, offspring were housed individually in opaque tubs [57 (L) x 38 (W) x 32 (H) cm], kept under a 25 W basking light set to a 0800-1800 h night/day cycle. Each tub was provided with cat litter as a substrate and a rock as a basking site and a refuge. Water was provided ad libitum.

Cognitive tasks

Offspring underwent two cognitive tasks: a foraging task based on colour associations ('foraging task') and an anti-predatory association task based on spatial associations ('anti-predatory', see below). Offspring were pseudo-randomly allocated into two batches (i.e., siblings were kept in the same batch) in order to randomize which cognitive task they began first. Our sample size was 72 offspring ('pre-birth– post-birth': high-high, n = 23; high-low, n = 16; low-high, n = 17; low-low, n = 16). The experimenters conducting and scoring the tasks were blind to the treatment of the offspring.

Foraging task: Offspring had to learn to associate a food reward with a specific colored block. This task is based on cognitive tasks previously used with lizards [5-7]. Specifically, each task consisted of a blue and a white block of wood [7 (L) x 7 (W) x 4 (H) cm] being placed in front of the offspring, one of which allowed access to a mealworm (the 'correct' block) while the other prevented access. Specifically, a petri dish (6 cm diameter) was placed on top of each block using Bluetak® and was used as a food-well. We placed a mealworm in both of the dishes to control for chemical and auditory cues, but blocked access to the mealworm on the 'incorrect' block by placing a mesh screen inside the dish. We randomized the colour of the 'correct' block between offspring. We also randomized the position and colour of the two blocks between trials so that a specific block was never used more than twice in the same position to account for potential differences in colour and spatial bias between offspring. Each dish was wrapped in black, opaque tape and elevated to ensure that offspring could not see the mealworm during the experiment.

We began each trial by placing the two blocks in front of the offspring. Each offspring was then given a maximum of 1 h to attempt the task. Each offspring was assayed twice daily, in the morning between 0900 and 1000 and in the afternoon between 1300 and 1400 over a 10-day period (n = 20 trials per offspring). The number of trials given to each offspring was based on protocols used successfully in previous studies on lizards [7, 8]. All trials were conducted in the offspring's enclosure and were video recorded using a CCTV system (H.264 DVR with Sony 1/2" high resolution colour cameras). For each trial, we scored the offspring's first choice (i.e., correct vs incorrect). We considered an offspring to have made a choice if it placed both its forelimbs on the top edge of a block. If an offspring did not choose any of the blocks we marked it as having made no choice. A single experimenter (KLM) conducted and scored all trials for this task.

In an attempt to reduce any effect of satiation on our results we did not providing any additional food during this task. The only food offspring received was the food reward (1 mini mealworm) on offer twice daily if they successfully completing the task (i.e., choose the correct colour block).

Anti-predatory task: We set up a biologically relevant anti-predatory assay, which has been used with success in previous lizard studies [9, 10]. During this task, offspring had to learn the location of a 'safe' refuge when given the choice of two refuge options (the safe refuge and an unsafe refuge; see below for definition of safe and unsafe). We kept the location of both refuges constant throughout the trials but randomized the location of which refuge was 'safe' and 'unsafe' between offspring. To help the offspring spatially orientate itself we added a strip of yellow plastic tape on one of the inner walls of the enclosure to act as an intra-maze cue.

All trials were conducted in the offspring's home enclosure. At the beginning of each trial we removed the water dish and basking rock in the offspring's enclosure and mixed up the litter substrate. We then placed the offspring under a central shelter and added the two 'refuge' options (11 cm diameter Terracotta saucer with an opening on one side) at opposite ends of the enclosure. We removed the central shelter shielding the offspring and gave each offspring five minutes to acclimate before the anti-predatory assay began (see below for how we dealt with instances where offspring were found inside a refuge at the end of the acclimation period). After this time, we simulated a predatory attack

following a standardized protocol by chasing the offspring around its enclosure by gently tapping its pelvic girdle from behind with a latex gloved index finger until it entered the 'safe' refuge. If the offspring entered the 'unsafe' refuge, we then lifted and returned the refuge and resumed chasing the offspring. We gave an offspring a maximum of 10 min (post the acclimation period) to attempt the task. All offspring were assayed daily for 10 days (n = 20 trials per offspring) during the active period of the day (1000-1500) and with at least 2 h between trials. Previous studies on lizards have shown this to be an appropriate number of trials to tease apart individual differences in learning [9]. Three experimenters (KLM, IK, LVG) helped conduct the task but were randomly allocated offspring each trial.

As with the previous task, we measured which refuge the offspring choose first (i.e., the correct 'safe' refuge or the incorrect 'unsafe' refuge). We considered an offspring to have made a choice if it ran inside either refuge. If an offspring was found inside the 'safe' refuge prior to the end of the acclimation period, we considered it as having made a correct choice. We argue that this is a biologically sensible interpretation, as we expect offspring would learn the location of the safe 'refuge' over time and take refuge. However, we acknowledge that this scoring method might inflate the number of correct choices so we ensured that there were no differences between the different resource treatments in number of times of offspring were found in the 'safe' refuge at the beginning of the trial (Likelihood ratio test (LRT), $\chi^2 = 0.09$, $p = 0.76$; Table S2.1).

If an offspring were found inside the 'unsafe' refuge prior to the end of the acclimation period, we considered it to have chosen incorrect and began the predatory attacks. If an offspring did not choose any refuge during the trial we gently forced it into the 'safe' refuge but scored this as an incorrect choice. At the completion of each trial we left the 'safe' refuge in the enclosure for a further 30 min but added the central shelter, water dish and basking rock.

Chemical cues are expected to play an important role in influencing lizards' decisions [11]. We are confident that we were able to control for offspring's use of chemosensory cues due to following measures; for both tasks we mixed cage substrate prior to each trial, we also washed blocks/refuges with 80% ethanol immediately after each trial, and for the anti-predatory task we alternated which refuge was used as 'safe' and 'unsafe' in

each trial for each offspring. In addition, we did not observe offspring tongue flicking during the trials for either of the tasks, suggesting that they were not utilizing chemical cues to locate the correct options.

At the completion of the learning tasks (early May), all lizards (mothers and offspring) were released back into the natural populations.

Statistical analysis

We modelled decision-making (probability of choosing correct – intercept on average trial) and learning rates (probability of choosing correct across trials) using generalized linear mixed models (GLMMs) in the 'lme4' package [12] in R v 3.0.3 [13]. We modelled offspring's probability of choosing correct [i.e., 'first choice' ('1' = correct; '0' = incorrect)] using a Bernoulli error distribution ('logit' link) for each task respectively. If lizards were learning, we predicted that the probability of making correct choices would increase across trials providing evidence that individuals were more likely to choose the correct dish. We initially fitted our models with pre- and post-birth treatments and trial as fixed effects. We also examined interactions between the resource treatments and trial to test for differences in learning rate across trials between the treatments but removed the interactions and refit the model excluding these interactions as they were not significant. To control for non-independence of offspring's previous experience with the task, we estimated a random slope (i.e., trial) and intercept for each offspring (i.e., a random regression model). However, model comparison indicated that models had better convergence and were more conservative with offspring identity as a random effect only. We also fitted a random intercept for maternal identity to account for non-independence of siblings. We included snout-vent length (SVL) at 6 weeks as a covariate to account for any differences in body size between the resource treatments that may influence learning or motivation [14]. Indeed, changes in offspring SVL growth from birth to the end of the experiment (i.e., 6 weeks of age) differed significantly between the post-birth resource treatments [ANOVA: $F_{1,34.38} = 9.14$, $P < 0.01$; Botterill-James *et al.* in revision for full discussion]. Here, we use SVL rather than mass as it is more widely used in reptile cognitive studies [1, 7, 14]. Continuous variables (i.e., SVL and trial) were scaled prior to analysis by mean centring and dividing by their standard deviation to improve model interpretation [15]. In all models on the anti-predatory task we initially

included experimenter identity as a fixed effect to account for any differences between the three experimenters conducting trials; this did not impact the result (*LRT*, χ^2 = 0.58, p = 0.75; Table S2.2), and so, we present the reduced model without this variable.

We assessed the adjusted repeatability of offspring's decisions using a Binary distribution (logit link) in the 'rptR' package [16]. We included z-transformed trial as a confounding variable, and offspring identity as a random effect and grouping variable. We first ran the models including all offspring, irrespective of pre-birth treatment, and then for each pre-birth treatment separately to determine how much of the variation in our data could be attributed to the effect of pre-birth treatment.

We tested for lateralization bias using GLMMs in the 'lme4' package [12]. We modelled the probability of offspring choosing the dish/shelter on their right side first ('1' = right, '0' = left), as a function of the independent variables, z-transformed SVL, trial, and pre-birth treatment. We also included individual identity and maternal identity as random effects

Extended results

Foraging task

Pre-birth resource treatment had a significant effect on an offspring's probability of choosing correct. Offspring that developed under low resource conditions were significantly less likely to choose the correct dish (*LRT*, χ^2 = 4.90, p = 0.03). This did not change across trials (trial × pre-birth, *LRT*, χ^2 = 0.182, p = 0.67) indicating that it was a decision bias rather than learning *per se*. We found no evidence that post-birth treatment influenced offspring decisions (*LRT*, χ^2 = 0.002, p = 0.97) or learning rates (trial × post-birth: *LRT*, χ^2 = 0.03, p = 0.85). Nor was there any evidence for an interaction between pre- and post-birth treatment on offspring decisions (pre- × post-birth, *LRT*, χ^2 = 0.01, p = 0.92) or learning rates (trial × pre- × post-birth, *LRT*, χ^2 = 0.69, p = 0.41).

There was limited evidence that offspring, irrespective of pre-birth treatment, were repeatable in their decision-making (R_{adj} = 0.06, CI = 0.02-0.09, $p < 0.001$). Furthermore, there was no evidence that differences in decision-making was caused by lateralization

bias between the pre-birth treatment groups (*LRT*, χ^2 = 0.49, *p* = 0.48; Table S2.3), or was driven by maternal effects (beyond that of pre-birth treatment) (*LRT*, χ^2 = 1.00, *p* = 0.32). Together this suggests that a larger proportion of variation in decision-making is the result of the specific pre-natal treatment offspring are born from.

Anti-predatory task

Pre-birth resource treatment had a significant effect on an offspring's probability of choosing correct. Offspring developed under low resource conditions were more likely to choose the correct dish (*LRT*, χ^2 = 4.51, *p* = 0.04). As with the foraging task this did not change across trials (trial × pre-birth: *LRT*, χ^2 = 1.08, *p* = 0.30), confirming that the cognitive process affected was decision-making rather than learning. Again, post-birth treatment did not influence offspring decisions (*LRT*, χ^2 = 0.42, *p* = 0.52) or learning rates (trial × post-birth: *LRT*, χ^2 = -0.0002, *p* = 0.99). Nor was there any evidence for an interaction between pre- and post-birth treatment on offspring decisions (pre- × post-birth: *LRT*, χ^2 = 0.42, *p* = 0.52) or learning rates (trial × pre- × post-birth: *LRT*, χ^2 = 1.90, *p* = 0.17).

As with the previous task, we found no evidence that offspring, irrespective of pre-birth treatment, were repeatable in their decision-making (R_{adj} = 0.00, CI = 0-0.02, *p* = 1). Nor was there any evidence that differences in decision-making was caused by lateralization bias between the pre-birth treatments (*LRT*, χ^2 = 0.22, *p* = 0.64; Table S2.3), or was driven by maternal effects (beyond that of pre-birth treatment) (*LRT*, χ^2 = 0.00, *p* = 1). As with the previous task, taken together this suggests that the pre-birth treatment accounted for most of the variation in our data.

Exploring the lack of learning

Our inability to detect learning in either the foraging task or anti-predatory could be due to a non-linear effect of trial; that is, learning could have occurred early in the trial process only to plateau or decrease due to 'over-training' (see [10] for similar effects on latency). We therefore decided to model a quadratic term for trial effects for both the foraging and anti-predatory tasks.

Foraging task: We found evidence of a non-linear effect of trial with offspring showing an initial increase in their probability of choosing correct early in the trial process, only to significant decrease their number of correct choices in the second half ($^{\sigma}$trial, χ^2 = 11.37, p = 0.001; Table S2.4, Figure S2.1). This pattern was not influenced by offspring's pre-birth treatment (trial × pre-birth, χ^2 = 0.15, p = 0.70; $^{\sigma}$trial × pre-birth, χ^2 = 0.07, p = 0.80). Following this result, we limited our analysis to only include the first 12 trials and found that offspring significantly increased their probability of choosing correct across the 12 trials (χ^2 = 9.18, p = 0.002; Table S2.5; Figure S2.2). Again, this pattern was not influenced by offspring's pre-birth treatment (χ^2 = 0.01, p = 0.92). Together this support the hypothesis that over-training occurred in the foraging task.

Anti-predatory task: We found no evidence that trial effect was non-linearly related to the probability of choosing correct (χ = 0.11, p = 0.73; $^{\sigma}$trial, χ^2 = 0.36, p = 0.55; Table S2.4; Figure S2.1). Nor was this influenced by offspring's pre-birth treatment (trial × pre-birth, χ^2 = 1.09, p = 0.30; $^{\sigma}$trial × pre-birth, χ^2 = 0.78, p = 0.38). This may suggest that learning manifested itself in some other way within this trial that we were unable to detect in this study.

Table S2.1 Parameter estimates and 95% confidence intervals from GLMMs examining effect of pre- and post-birth resource availability (high vs low) on the number of trials were offspring were found in the 'safe' refuge at the beginning of the anti-predatory task (N_{obs} = 1440, $N_{lizards}$ = 72).

	estimate	**lower CI**	**upper CI**	***p*-value**
Intercept	**-2.02**	**-2.48**	**-1.63**	**<0.001**
Scaled SVL	-0.16	-0.43	0.11	0.23
Scaled trial number	0.12	-0.03	0.27	0.10
Treatment pre-birth LOW	0.06	-0.48	0.58	0.83
Treatment post-birth LOW	0.21	-0.27	0.74	0.39
Pre - × post-birth	0.53	-0.47	1.56	0.29

Statistically significant results are in bold. Main effects are presented from a model without interactions.

Table S2.2 Parameter estimates and 95% CI of GLMMs the relationship between pre- and post-birth resource availability on offspring's probability of choosing correct in an anti-predatory task including experimenter identity as a main effect in the models.

	estimate	**lower CI**	**upper CI**	***p*-value**
Intercept	0.01	-0.22	0.25	0.90
Scaled SVL	-0.10	-0.21	0.01	0.09
Scaled trial number	0.02	-0.08	0.13	0.67
Treatment pre-birth LOW	**0.25**	**0.02**	**0.47**	**0.03**
Treatment post-birth LOW	-0.07	-0.28	0.14	0.53
Handler 2	-0.08	-0.32	0.17	0.52
Handler 3	-0.11	-0.44	0.21	0.49
Scaled trial × pre-birth	0.11	-0.10	0.32	0.30
Scaled trial × post-birth	0.004	-0.21	0.21	0.97
Pre- × post-birth	-0.13	-0.56	0.29	0.54

Statistically significant results are in bold. Main effects are presented from a model without interactions.

Table S2.3 Parameter estimates and 95% confidence intervals from GLMMs examining the effect of pre-birth resource availability (high vs low) on the lateralization (choice of left or right) in a foraging and anti-predatory task (foraging: N_{obs} = 1257, $N_{lizards}$ = 71, anti-predatory: N_{obs} = 1440, $N_{lizards}$ = 72).

	Foraging task				Anti-predatory task			
	estimate	lower CI	upper CI	*p*-value	estimate	lower CI	upper CI	*p*-value
Intercept	0.20	-0.11	0.50	0.19	0.04	-0.10	0.19	0.58
Scaled SVL	-0.05	-0.28	0.18	0.67	0.02	-0.09	0.16	0.79
Scaled trial number	0.06	-0.06	0.18	0.34	0.03	-0.07	0.13	0.60
Treatment pre-birth LOW	-0.16	-0.61	0.30	0.48	-0.05	-0.27	0.17	0.64

There were no statistically significant results.

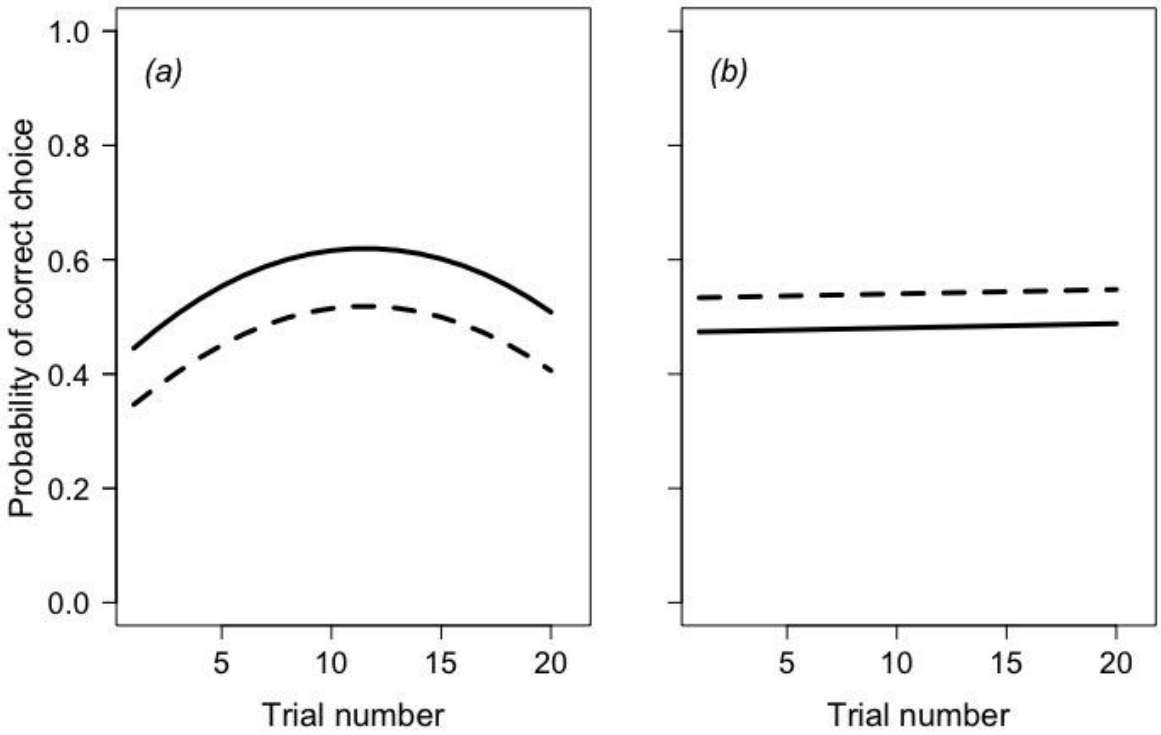

Figure S2.1 Predicted probability of learning (*a*) a foraging and (*b*) an anti-predatory task across 20 trials for offspring developed under high (solid line) or low (dashed line) resource availability pre-birth. Predicted probabilities are based on best fit models resulting in trial effect being modelled as a non-linear response for the (*a*) foraging task, but not the anti-predatory task.

Table S2.4 Parameter estimates and 95% confidence intervals (CI) of GLMMs examining the relationship between pre-birth resource availability on offspring's probability of choosing correct in a foraging and anti-predatory task with trial effect modelled as non-linear.

	Foraging task				Anti-predatory task			
	estimate	lower CI	upper CI	*p*-value	estimate	lower CI	upper CI	*p*-value
Intercept	0.47	0.16	0.79	0.003	-0.08	-0.29	0.12	0.42
Scaled SVL	0.22	0.05	0.39	0.01	-0.10	-0.21	0.01	0.08
Scaled trial number	0.08	-0.04	0.19	0.18	0.02	-0.09	0.12	0.73
$^{\sigma}$ Scaled trial number	**-0.22**	**-0.35**	**-0.09**	**<0.001**	0.04	-0.09	0.15	0.55
Treatment pre-birth LOW	**-0.42**	**-0.79**	**-0.05**	**0.02**	**0.25**	**0.02**	**0.47**	**0.03**
Treatment post-birth LOW	0.02	-0.33	0.37	0.92	-0.07	-0.28	0.14	0.52
Scaled trial × pre-birth	0.04	-0.18	0.27	0.70	0.11	-0.10	0.32	0.30
$^{\sigma}$ Scaled trial × pre-birth	0.03	-0.22	0.29	0.79	0.11	-0.13	0.34	0.38

Statistically significant results are in bold. Main effects are presented from a reduced model as interactions were non-significant ($p > 0.05$).

Table S2.5 Parameter estimates and 95% confidence intervals (CI) of GLMMs examining the relationship between pre-birth resource availability on offspring's probability of choosing correct in a foraging task limiting our analysis to only include the first 12 trials (N_{obs} = 753, $N_{lizards}$ = 71).

	estimate	lower CI	upper CI	*p*-value
Intercept	0.23	-0.05	0.51	0.10
Scaled SVL	0.13	-0.05	0.31	0.16
Scaled trial number	**0.23**	**0.08**	**0.38**	**0.002**
Treatment pre-birth LOW	**-0.38**	**-0.74**	**-0.02**	**0.04**
Treatment post-birth LOW	0.02	-0.32	0.37	0.93
Scaled trial × pre-birth	0.02	-0.28	0.31	0.92

Statistically significant results are in bold. Main effects are presented from a reduced model as interactions were non-significant ($p > 0.05$).

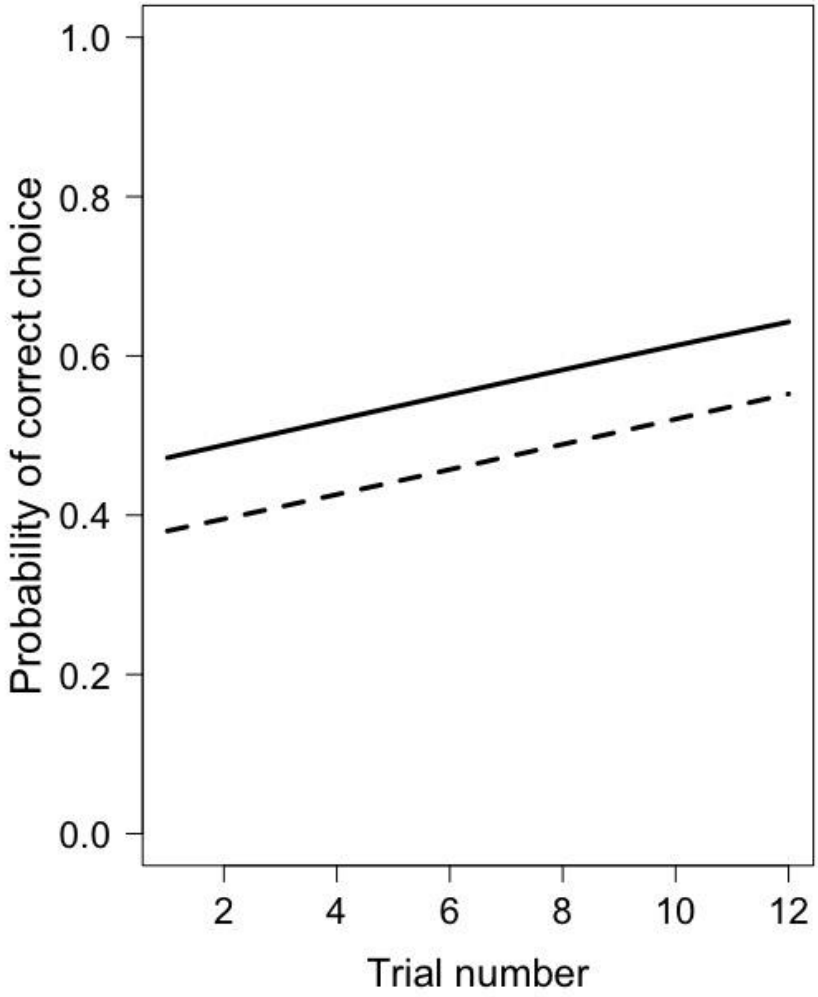

Figure S2.2 Predicted probability of learning a foraging task across 12 trials for offspring developed under high (solid line) or low (dashed line) resource availability pre-birth.

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